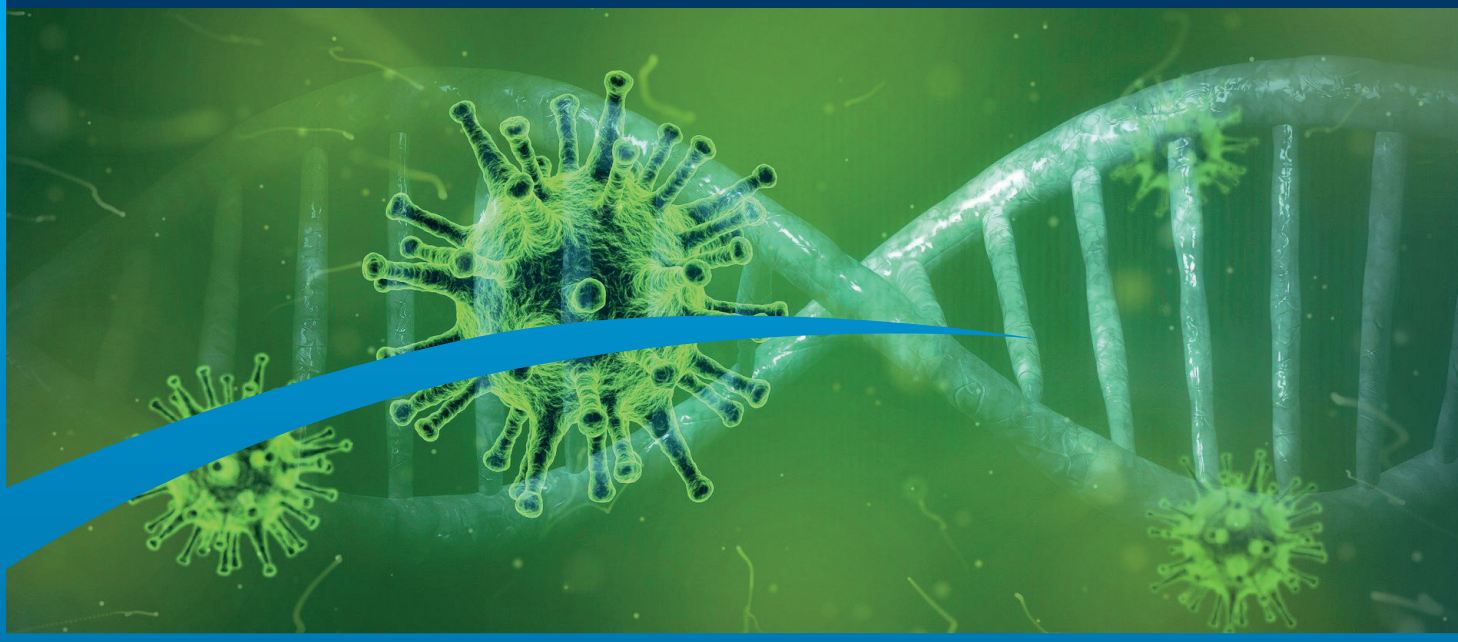
Medici oggi

ebookecm.it



Infezione da nuovo Coronavirus: notizie dalla letteratura e materiale informativo

Responsabile Scientifico: dr. Alberto Enrico Maraolo

Springer Healthcare Education

ebookecm.it

Questa pubblicazione è **accreditata ECM** solo su www.ebookecm.it

Questa pubblicazione è accreditata per **26 crediti ECM** per tutte le professioni, fino al 31/12/2020 (codice ECM 4146-294415).

Per accedere al corso ECM è necessario iscriversi dalla pagina
https://www.ebookecm.it/corsi-ecm-fad/corso-ebook-coronavirus-covid19-255.html

Il corso ECM basato su questa pubblicazione ha un costo di 69€ + IVA.

Medici Oggi

Infezione da nuovo Coronavirus: notizie dalla letteratura e materiale informativo

ISSN 1721-0208

Springer Healthcare Education

Via Decembrio, 28
20137 Milano

www.medicioggi.it
www.springerhealthcare.it

Registrazione del Tribunale di Milano n. 327 del 31/05/97
Direttore responsabile: Fabrizio Gervasoni

Indice

Articoli pubblicati su Medici Oggi, rivista di Springer Italia

Oltre il Coronavirus: surriscaldamento globale, urbanizzazione e globalizzazione alle radici dell'epidemia. Una nuova FAD ECM
A. Gallo 1

COVID-19: Italia in prima linea contro il nuovo Coronavirus
A. E. Maraolo 5

Coronavirus, facciamo chiarezza
Intervista al dottor Alberto Enrico Maraolo, Specialista in malattie infettive
A. Giambelluca 8

Infezione da Coronavirus 2019-nCoV – il mondo con il fiato sospeso
A. E. Maraolo 11

Coronavirus 2019-nCoV – dati incerti sui contagi in gravidanza
E. Finale 14

Diabete mellito, infezioni respiratorie e Sindrome COVID-19: evidenze disponibili e consigli pratici per la gestione delle persone con diabete durante il ricovero ospedaliero
C. Parrino 16

Occhi puntati al Coronavirus – Congiuntivite virale da COVID-19
P. Giorno 20

COVID-19: quali indicazioni nell'attuale incertezza scientifica per salvaguardare la gravidanza, il parto e l'allattamento?
E. Finale et al. 21

Articoli full text open access di vari editori in inglese

Commento in italiano a cura di A. E. Maraolo

PLOS Medicine - ***SARS: Systematic Review of Treatment Effects***
L. J. Stockman et al. 25

Journal of Global Health - ***Communicable disease control in China: From Mao to now***
D. Hipgrave 33

Viruses - ***Return of the Coronavirus: 2019-nCoV***
L.E. Gralinski et al. 49

Nature - ***A pneumonia outbreak associated with a new coronavirus of probable bat origin***
Zhou P. et al. 58

Military Medical Research - ***A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version)***
Jin JH P et al. 82

Journal of Clinical Medicine - ***Novel Coronavirus Outbreak in Wuhan, China, 2020: Intense Surveillance Is Vital for Preventing Sustained Transmission in New Locations***
Thompson R. N. 106

npj Vaccines - ***The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines***
Shang W. et al. 114

One Health Outlook - ***Implementing One Health approaches to confront emerging and re-emerging zoonotic disease threats: lessons from PREDICT***
Kelly T.R. et al. 118

Cell Research - ***Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro***
Wang M. et al. 126

Lancet Digital Health - ***Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population level observational study***
Sun K. et al. 130

Medici oggi

Oltre il Coronavirus: surriscaldamento globale, urbanizzazione e globalizzazione alle radici dell'epidemia. Una nuova FAD ECM

Alessandro Gallo

L'emergenza Coronavirus sembra essere un caso senza precedenti e praticamente nessuno sembra aver mai vissuto una situazione simile. Tuttavia, il colera, la peste bubbonica, il vaiolo e l'influenza rappresentano da secoli le malattie infettive più mortifere nella storia dell'umanità [1].

SARS, influenza suina e EBOLA

La SARS [2], l'influenza suina (swine flu) [3], l'Ebola, che si sono diffuse nelle ultime due decadi, sono conosciute soprattutto dagli addetti ai lavori e, pur essendo estremamente pericolose e letali, hanno avuto un impatto limitato in termini mediatici in Italia e in Europa.

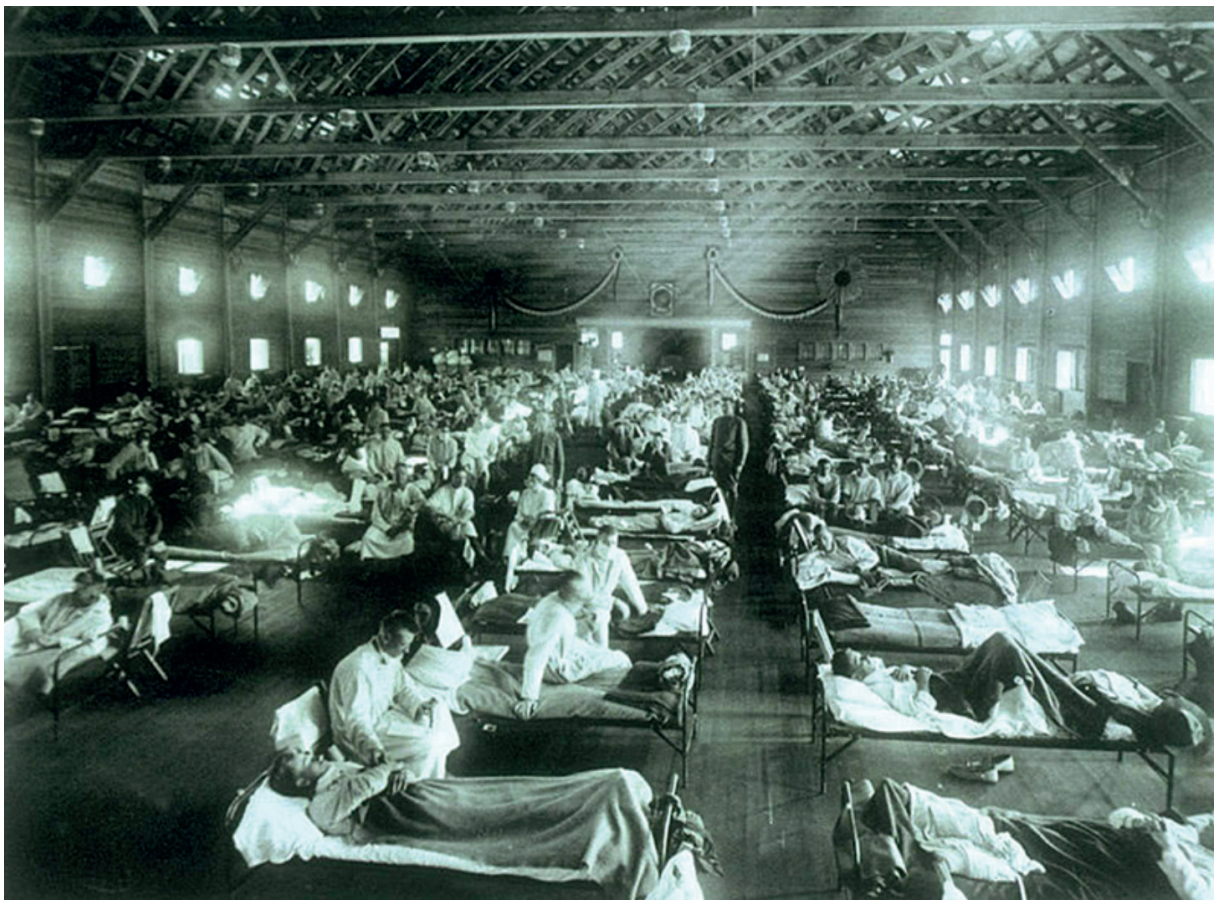


Figura 1. (Image: courtesy of the National Museum of Health and Medicine, Armed Forces Institute of Pathology, Washington, D.C., United States) CC BY (https://creativecommons.org/licenses/by/2.5)

La "Spagnola"

In passato alcune pandemie hanno causato un numero di morti nettamente superiore a quello di un conflitto bellico. Ad esempio la pandemia di influenza aviaria (bird flu) [4] che ha avuto luogo nel 1918-1919 [5] subito dopo la conclusione della Prima Guerra Mondiale, tristemente nota come "la spagnola" sembra aver fatto circa 50 milioni di morti e contagiato circa 500 milioni di persone, con un numero di decessi superiore a quelli causati dal conflitto bellico [6].
Da quando è cominciata l'emergenza Coronavirus in Cina, i governi hanno reagito con le classiche misure messe in atto in caso di epidemie, ovvero quarantena, limitazione di viaggi e mobilità delle popolazioni coinvolte nelle aree focolaio, con una corsa alla realizzazione di un vaccino.

Diversamente dal passato, tuttavia, questa è un'emergenza che mette a rischio la salute pubblica in un mondo globalizzato e interconnesso come mai prima d'ora. La malattia infettiva, infatti, si è sviluppata e propagata in un contesto economico e socio-culturale, con popolazioni progressivamente sempre meno rurali [7] che si addensano in contesti urbani con problemi di sovraffollamento non soltanto nei paesi in via di sviluppo [8].

L'eradicazione del vaiolo

Nel dicembre del 1979 l'OMS [9] ha proclamato l'eradicazione del vaiolo, annunciandone trionfalmente la scomparsa. Il vaiolo, che alcuni fanno risalire addirittura a 10.000 prima di Cristo [10], ha sterminato fino al XX secolo milioni di persone in tutto il mondo. Tuttavia, proprio a metà anni '70, mentre l'eradicazione del vaiolo stava avanzando, un'altra malattia infettiva letale ha cominciato a propagarsi l'Ebola [11]. Il vaiolo è finora l'unica malattia infettiva completamente debellata e quindi estinta.

La "zoonosi" il "salto" dagli animali agli esseri umani

Altre malattie continuano a "fare il salto" dagli animali agli esseri umani [12] (zoonosi). Malattie infettive di tipo respiratorio quali la SARS, che è in grado di passare facilmente dagli animali agli esseri umani [13], rendono sostanzialmente quasi impossibile una eradicazione definitiva. La peste che ha sterminato decine di milioni di persone in Europa nel XIV secolo [14], trasmessa attraverso i topi, è tuttora esistente in alcuni continenti [15].
Nel 2009, l'OMS ha dichiarato un'emergenza pandemica globale per l'H1N1 [16], altrimenti nota come febbre suina (swine flu), malattia che si è diffusa trasferendosi dai maiali all'uomo. Il CDC americano [17] ha stimato fino a 575.000 decessi da H1N1 nel primo anno (2009).

Urbanizzazione, allevamenti intensivi e surriscaldamento globale

L'industrializzazione degli allevamenti intensivi e l'urbanizzazione hanno notevolmente favorito la diffusione di alcune malattie infettive [18]. Gli allevamenti intensivi di bestiame, data la promiscuità, favoriscono la diffusione del contagio tra animali. Inoltre, il surriscaldamento globale favorisce una diffusione più ampia di alcune malattie infettive, ad esempio quelle trasmesse attraverso le zanzare, che con temperature inferiori in passato non avrebbero rappresentato un problema [19]. Secondo l'OMS, un aumento medio del-

la temperatura di 2-3 gradi comporterebbe un incremento del 3-5% del rischio di esposizione alla malaria in alcune geografie (alcune centinaia di milioni di persone [20]).
Inoltre, l'urbanizzazione sistematica e progressiva con condizioni abitative di sempre maggiore promiscuità favoriscono la diffusione rapida del contagio. La globalizzazione, il turismo d'affari e di piacere, i movimenti migratori con la possibilità per milioni di persone di spostarsi rapidamente e imprevedibilmente su base planetaria [21] rappresentano inoltre un altro elemento di importante diffusione di malattie infettive.

Altre emergenze sanitarie in corso: H5N1 in Cina, H1N1 a Taiwan, febbre suina africana in Indonesia

Le misure restrittive che hanno visto molti governi imporre quarantena e restrizioni alle popolazioni locali colpite dal contagio, sia in Cina che in Italia, sono mirate a contenere la diffusione e a rallentare l'afflusso di malati negli ospedali, ma non sono risolutive. In parallelo, proprio durante l'emergenza coronavirus, sono in corso un'epidemia di H5N1 [22], il cui focolaio è in una fattoria di pollame in Cina, di H1N1 a Taiwan [23], nonché di febbre suina africana in Indonesia [24] tutte apparentemente di rilevante importanza, che però non hanno avuto la stessa ricaduta mediatica.

L'impatto dell'uomo sull'ambiente circostante e sugli animali: l'approccio One Health

Riteniamo che sia necessario un approccio integrato per combattere le malattie infettive su scala globale. Negli ultimi anni si è parlato molto infatti di "One Health" [25]: un approccio "olistico" alla salute umana, da intendersi fortemente interconnessa con quella animale. Il comportamento umano e le interazioni con gli animali possono enormemente influire sulla diffusione delle malattie infettive. Le epidemie di malattie infettive non dipendono unicamente dall'efficacia delle vaccinazioni e dalle condizioni igienico-sanitarie. Per poter ridurre i rischi di esposizione a malattie infettive potenzialmente imprevedibili e sempre più virulente su scala globale, è necessaria una più corretta comprensione delle interazioni e dell'impatto che gli esseri umani hanno sull'ambiente circostante, sugli altri esseri viventi e sulle modalità attraverso le quali l'uomo sfrutta e "consuma" le risorse del pianeta.

La raccolta FAD ECM sul Coronavirus a cura di Alberto Maraolo

In questo contesto Springer Healthcare, in collaborazione con Bookia SRL e grazie al coordinamento scientifico del nostro section editor per le malattie infettive della rivista Medici Oggi dr. Alberto Maraolo, si appresta a pubblicare attraverso il portale www.ebookecm.it una FAD ECM sul Coronavirus. La raccolta di articoli, pubblicati su Medici Oggi (e altre importanti riviste internazionali e di utilissimi documenti diffusi da importanti organizzazioni nazionali e internazionali) viene incontro all'esigenza di fornire uno strumento per un autoaggiornamento efficace per il professionista sanitario, non basato esclusivamente sull'attualità del momento, ma incentrato anche sulla conoscenza e le esperienze del passato, nell'ottica di una formazione orientata verso il futuro, basata sulla programmazione e prevenzione e non solo sulla reazione a situazioni di crisi e emergenza.

Bibliografia

[1] Tognotti, Eugenia. "Lessons from the history of quarantine, from plague to influenza A." Emerging infectious diseases vol. 19,2 (2013): 254-9

[2] LeDuc, James W., and M. Anita Barry. "SARS, the First Pandemic of the 21st Century." Emerging Infectious Diseases vol. 10,11 (2004): e26.

[3] Gibbs, A.J., Armstrong, J.S. & Downie, J.C. "From where did the 2009 'swine-origin' influenza A virus (H1N1) emerge?" Virology Journal vol. 6 (2009) 207

[4] Lycett, Samantha J et al. "A brief history of bird flu." Philosophical transactions of the Royal Society of London. Series B, Biological sciences vol. 374,1775 (2019)

[5] Lycett, Samantha J et al. "A brief history of bird flu." Philosophical transactions of the Royal Society of London. Series B, Biological sciences vol. 374,1775 (2019): 20180257

[6] Martini, M et al. "The Spanish Influenza Pandemic: a lesson from history 100 years after 1918." Journal of preventive medicine and hygiene vol. 60,1 E64-E67. 29 Mar. 2019

[7] Andrew P. Dobson, E. Robin Carper, Infectious Diseases and Human Population History: Throughout history the establishment of disease has been a side effect of the growth of civilization, BioScience, Volume 46, Issue 2, February 1996, Pages 115–126

[8] Hipgrave, David. "Communicable disease control in China: From Mao to now." Journal of global health vol. 1,2 (2011): 224-38.

[9] Wehrle, Paul F. "A Reality in Our Time: Certification of the Global Eradication of Smallpox." The Journal of Infectious Diseases, vol. 142, no. 4, 1980, pp. 636–638

[10] Riedel, Stefan. "Edward Jenner and the history of smallpox and vaccination." Proceedings (Baylor University. Medical Center) vol. 18,1 (2005): 21-5

[11] Awah, Paschal Kum et al. "Ebola Virus Diseases in Africa: a commentary on its history, local and global context." The Pan African medical journal vol. 22 Suppl 1, 18. 11 Oct. 2015

[12] Bidaisee, Satesh, and Calum N L Macpherson. "Zoonoses and one health: a review of the literature." Journal of parasitology research vol. 2014 (2014)

[13] Sheahan, Timothy et al. "Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium." Journal of virology vol. 82,5 (2008): 2274-85

[14] DeWitte SN "Mortality Risk and Survival in the Aftermath of the Medieval Black Death". PLoS ONE 9(5) (2004) e96513.

[15] Mead, Paul "Epidemics of plague past, present, and future" Lancet Infectious Diseases Volume 19 (2019) Issue 5, P459-460

[16] Fineberg, H "Pandemic Preparedness and Response — Lessons from the H1N1 Influenza of 2009" in NEJM (2014) 370:1335-1342

[17] US Centers for Disease Control and Prevention https://www.cdc.gov/flu/pandemic-resources/2009-h1n1-pandemic.html ultimo accesso 2 marzo 2020

[18] Jones, Bryony A et al. "Zoonosis emergence linked to agricultural intensification and environmental change." Proceedings of the National Academy of Sciences of the United States of America vol. 110,21 (2013): 8399-404.

[19] Irfain, U. "As Temperatures Climb, So Does Malaria. The risk area for malaria

expanded between 1990 and 2005" in Scientific American 2014 https://www.scientificamerican.com/article/as-temperatures-climb-so-does-malaria ultimo accesso 2 marzo 2020

[20] World Health Organisation, Climate Change and Human Health https://www.who.int/globalchange/climate/summary/en/index5.html ultimo accesso 2 marzo 2020

[21] Jan C Semenza, PhD, Kristie L Ebi, PhD, Climate change impact on migration, travel, travel destinations and the tourism industry, Journal of Travel Medicine, Volume 26, Issue 5, 2019, taz026,

[22] Zhou, C. "China reports outbreak of deadly bird flu among chickens in Hunan province, close to coronavirus epicentre of Wuhan" in South China Morning Post, 2 febbraio 2020 https://www.scmp.com/news/china/society/article/3048566/china-reports-outbreak-deadly-bird-flu-among-chickens-hunan – ultimo accesso 2 marzo 2020

[23] Taiwan News "H1N1 flu virus kills 13 people in Taiwan in one week" 2 febbraio 2020 https://www.taiwannews.com.tw/en/news/3868793 – ultimo accesso 2 marzo 2020

[24] Komang Erviani "Bali confirms swine fever outbreak amid numerous pig deaths" in The Jakarta Post 5 febbraio 2020 https://www.thejakartapost.com/news/2020/02/05/bali-confirms-swine-fever-outbreak-amid-numerous-pig-deaths.html, ultimo accesso 2 marzo 2020

[25] Bidaisee, Satesh, and Calum N L Macpherson. "Zoonoses and one health: a review of the literature." Journal of parasitology research vol. 2014 (2014)

COVID-19: Italia in prima linea contro il nuovo Coronavirus

Alberto Enrico Maraolo

Controversie e questioni aperte sul territorio nazionale connesse all'infezione da SARS-COV-2

21 Febbraio 2020: l'Italia "scopre" il coronavirus

La mattina di venerdì 21 febbraio l'Italia si è svegliata iniziando a conoscere i particolari di colui che è stato definito il "paziente 1": un uomo di 38 anni, proveniente da Castiglione d'Adda nel lodigiano, accorso all'ospedale di Codogno il giorno prima per gravi difficoltà respiratorie. La diagnosi arrivata in serata era stata clamorosa: infezione da **nuovo coronavirus**, ribattezzato ormai come **SARS-COV-2** (*severe acute respiratory syndrome coronavirus 2*), e responsabile della sindrome nota come **COVID-19** (*coronavirus disease* 2019).

Il sospetto diagnostico era nato per un'importante notizia anamnestica: il contatto con un manager italiano di ritorno dalla Cina, definito come "paziente zero". Tuttavia, nei giorni successivi lo scenario si è completamente modificato: non solo il "paziente zero" si è rivelato completamente negativo ad esposizioni al virus anche pregresse, valutate mediante indagini sierologiche (ricerca di anticorpi), ma

si è scoperta una diffusione del contagio estesa a molte aree del Nord Italia.
Si è dunque passati da una "fase 1", in cui il nuovo coronavirus sembrava un problema lontano da importazione, come accade per varie malattie esotiche, a una "fase 2" in cui l'Italia è il terzo paese al mondo per numero di infetti, dopo Cina e Corea del Sud.

I primi dieci giorni della "fase 2": bilancio al primo marzo 2020

Al primo marzo, i dati rilasciati dal Ministero della Salute sono i seguenti: 1577 persone hanno contratto il virus in 14 Regioni e in una Provincia autonoma (984 in Lombardia, 285 in Emilia-Romagna e 263 in Veneto, le tre aree più colpite). Di queste 1577, 83 risultano già guarite, 798 risultano in isolamento domiciliare (non manifestando sintomi o manifestandone di lieve entità), 779 sono ricoverate (di cui 140 in terapia intensiva, ossia il 9% circa dei casi diagnosticati); i decessi, riguardanti soprattutto soggetti anziani affetti da varie comorbidità, sono 34, con un *case-fatality rate* globale (la letalità) pari al 2,2%.
Tali numeri, unitamente a deduzioni sulla base dell'analisi filogenetica delle sequenze virali disponibili, fanno supporre una diffusione "silente" del virus sul territorio italiano già da settimane, probabilmente da gennaio, quando focolai di polmoniti atipiche nel basso lodigiano erano già stati segnalati, per esempio. D'altronde, vi sono vari indizi di una sostenuta trasmissione interumana già a inizio dicembre in Cina, per cui è verosimile che un gran numero di persone abbia "portato" SARS-COV-2 al di fuori dell'area di Wuhan ben prima che la nuova sindrome fosse riconosciuta. Peraltro, escludendo la Cina, l'Italia era al terzo posto, dopo Regno Unito e Germania, nella classifica di rischio di importazione del virus nella fase pre-quarantena di Wuhan secondo modelli matematici basati sul traffico aereo: non sorprendentemente, l'aeroporto italiano più esposto secondo tali modelli era Milano Malpensa.

COVID-19: controversie in Italia

L'esplosione in Nord Italia del numero di casi di COVID-19 in pochi giorni sta mettendo a dura prova il sistema sanitario, con intasamento di numerosi pronto soccorso e reparti di malattie infettive nonché di terapia intensiva, con ovvie ricadute sulla capacità del sistema di offrire adeguata assistenza a pazienti con problemi differenti.
Si sono create tensioni tra governo centrale e governi locali in merito alle misure da intraprendere, tra cui la più rilevante è stata il "cinturamento" dei comuni focolaio, la cosiddetta "zona rossa".
Dal punto di vista scientifico, la decisione più significativa è stata quella di stabilire la necessità di sottoporre a esame diagnostico per COVID-19 tramite tampone rino-faringeo solo soggetti sintomatici (con link epidemiologico). La deliberazione è avvenuta il 26 febbraio, dopo cinque giorni circa di intesa attività di screening a tappeto, che è stata utile per far emergere l'entità del problema, ma ha prodotto anche un'enorme quantità di falsi negativi che hanno ingolfato i laboratori. Allo stato attuale, il livello d'infettività nel corso delle fasi asintomatiche/prodromiche delle infezioni da SARS-CoV-2 non è compiutamente noto: comunque, il rischio di trasmissione virali in tali fasi sembra essere molto basso.
Non bisogna poi dimenticare che, come ogni test diagnostico, anche quello ad oggi in uso ha dei limiti in termini di sensibilità e specifi-

cità, e che la reale prevalenza della patologia (ovviamente ignota al momento) nelle varie aree geografiche influenza la predittività del test. Poiché sussiste il rischio di falsi positivi, tutti i casi devono essere confermati dall'Istituto Superiore di Sanità.

COVID-19: questioni aperte

Le domande ancora aperte sono numerose. Si riuscirà a ricostruire la catena del contagio in Italia? Vi è stato un primo focolaio o sono scoppiati più focolai indipendenti? Le misure di infection control intraprese includenti ordinanze ad alto impatto sociale (chiusura scuole, per esempio) avranno un effetto nel ridurre la diffusione del virus sul territorio nazionale? Il sistema sanitario nazionale reggerà nelle aree a maggior incidenza? Vi saranno problemi di ordine pubblico legati all'infodemia e alla psicosi collettiva? Fin quando continueranno misure restrittive nei confronti degli italiani all'estero?
Il nuovo coronavirus rappresenta sicuramente la più grande sfida per il sistema paese Italia del nuovo secolo dopo la crisi dello spread del 2011.

Bibliografia essenziale

- Carinci F. Covid-19: preparedness, decentralisation, and the hunt for patient zero. BMJ. 2020 Feb 28;368:bmj.m799. doi: 10.1136/bmj.m799.
- Lai Q et al. Early Phylogenetic Estimate of the Effective Reproduction Number Of Sars-CoV-2. J Med Virol. 2020 Feb 25. doi: 10.1002/jmv.25723. [Epub ahead of print]
- Nishiura et al. Initial Cluster of Novel Coronavirus (2019-nCoV) Infections in Wuhan, China Is Consistent with Substantial Human-to-Human Transmission. J Clin Med 2020; 9 pii: E488
- La Repubblica (a cura di Giampaolo Visetti). Coronavirus, polmoniti anomale a metà gennaio: "Così è nato il focolaio di Codogno". Last accessed 1 March 2020. https://www.repubblica.it/cronaca/2020/02/28/news/_polmoniti_anomale_a_meta_gennaio_cosi_e_nato_il_focolaio_di_codogno_-249759796/
- Ministero della Salute. Covid-19 – Situazione in Italia. Last accessed 1 March 2020. http://www.salute.gov.it/portale/nuovocoronavirus/dettaglioContenutiNuovoCoronavirus.jsp?lingua=italiano&id=5351&area=nuovoCoronavirus&menu=vuoto
- Ministero della Salute. Documento relativo ai criteri per sottoporre soggetti clinicamente asintomatici alla ricerca d'infezione da SARS-CoV-2 attraverso tampone rinofaringeo e test diagnostico (A cura del Gruppo di lavoro permanente costituito nell'ambito del Consiglio Superiore di Sanità) Last accessed 1 March 2020. http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2020&codLeg=73444&parte=1%20&serie=null
- Research on Complex Systems – Brockmann Lab. Event Horizon — COVID-19. Last accessed 1 March 2020. http://rocs.hu-berlin.de/corona/docs/analysis/importrisk/#pre-quarantine-risk-assessment
- WHO. Naming the coronavirus disease (COVID-19) and the virus that causes it. Last accessed 1 March 2020. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it

Coronavirus, facciamo chiarezza

Angelica Giambelluca

Intervista al dottor Alberto Enrico Maraolo, Specialista in malattie infettive

L'OMS ha già parlato di infodemia, vale a dire una diffusione massiccia e capillare di notizie, alcune delel quali false, inerenti il Coronavirus, capaci di generare eccessivo allarmismo, disinformazione e da ultimo, isteria.
L'epidemia da Coronavirus non va sottovalutata, ma prima di preoccuparsi eccessivamente per la propria salute o quella dei proprio cari, è bene conoscere l'argomento di cui stiamo parlando.
Non voglio aggiungere fiumi di parole all'infodemia di cui parla l'OMS ma solo chiarire, con cinque semplici domande, di che cosa stiamo parlando.
Lo faccio con il prezioso aiuto **Alberto Enrico Maraolo**, ricercatore medico di malattie infettive dell' **Università degli Studi di Napoli "Federico II"**

Partiamo dalla domanda più semplice: quali sono le differenze tra l'influenza causata dal Coronavirus e quella "comune" contro cui lottiamo tutti gli anni, soprattutto nel periodo invernale?
Iniziamo dalle analogie. Sia il nuovo coronavirus, chiamato 2019-nCoV, sia il virus influenzale (che include diversi sottotipi) possono causare patologie respiratorie di lieve o grave intensità e potenziali complicanze molto rilevanti. Non parliamo del raffreddore comune che, ricordiamo, può essere causato da alcuni tipi di coronavirus che circolano da tempo nella popolazione ma sono diversi da quello venuto recentemente alla ribalta.
Veniamo alle differenze.

Incubazione. L'influenza "tradizionale" ha in genere un periodo di incubazione brevissimo, di circa 2 giorni, mentre **per 2019-nCoV i valori medi sono più alti, ossia 5-6 giorni.**
Contagio. L'influenza può essere trasmessa da un soggetto asintomatico nelle 24 ore precedenti la comparsa dei sintomi, ma **il ruolo dei soggetti asintomatici nel caso di 2019-nCoV è ancora tutto da chiarire**, anche se, come afferma l'Organizzazione Mondiale della Sanità (OMS), essi sicuramente non sono il maggiore mezzo di diffusione del contagio.
Bambini. L'influenza "tradizionale" interessa molto spesso l'età pediatrica, causando, a seconda delle stime, anche fino a 100.000 decessi circa nei bambini al di sotto dei 5 anni ogni anno nel mondo, specialmente nei paesi in via di sviluppo. Invece, **2019-nCoV sembra risparmiare l'età pediatrica** o comunque causare nei soggetti più piccoli quadri clinici molto lievi.

È vero che è letale soprattutto su soggetti già debilitati e anziani?
Fin da quando l'epidemia scoppiata a Wuhan ha raggiunto notorietà mondiale, si è capito che l'infezione era capace di determinare forme cliniche gravi e anche mortali. La morte in genere avviene per grave insufficienza respiratoria a causa di un'importante compromissione della funzione polmonare.
Sono stati comunque descritti quadri di disfunzione anche di altri organi, insufficienza renale e danno cardiaco acuto. Nei casi più gravi il decesso può avvenire anche per sovrainfezioni batteriche.
I meccanismi che sono alla base della patolo-

gia ancora non sono del tutto chiariti, perché non si ha a disposizione una massa critica di dati tale da permettere di capire con ragionevole certezza fattori di rischio e categorie più vulnerabili. Tuttavia, dai primi report sembra che i pazienti più sfortunati siano quelli di età avanzata e che soffrono di diverse patologie contemporaneamente, la cosiddetta *comorbidità* (ad esempio, soffrire di diabete e patologie cardiovascolari). Ciò non sorprende perché avviene lo stesso per l'influenza.
Ovviamente questa è la visione d'insieme, relativa ai grandi numeri: **il singolo caso fa sempre storia a sé** e, sempre similmente all'influenza, un andamento aggressivo non può essere escluso a priori in ogni soggetto giovane e senza problemi di salute rilevanti, sebbene ciò sia generalmente poco probabile.

Se è vero che la mortalità, al momento, è allo stesso livello o poco più alta di una "banale" influenza (198 morti l'anno scorso solo in Italia, dati del Sole 24 Ore) perché è esplosa l'emergenza sanitaria a livello globale?
In realtà l'influenza "classica" è una malattia tutt'altro che "banale" come purtroppo è scolpita nell'immaginario collettivo. Le stime dell'OMS parlano di un numero di morti da 290.000 a 650.000 ogni anno nel mondo. I numeri ufficiali, come le poche centinaia di casi in Italia, rappresentano largamente una sottostima, legata al fatto che **l'influenza spesso non è la causa ultima del decesso, ma ne è il fattore scatenante.** È dimostrato come l'influenza possa innescare, in particolar modo in soggetti con pre-esistenti comorbidità, eventi cardiovascolari come l'infarto. Inoltre, sempre nei soggetti più fragili, è tutt'altro che rara l'ospedalizzazione a cui segue la comparsa, per esempio, di una complicanza come la polmonite nosocomiale (vale a dire che insorge in seguito al ricovero in ospedale) che può essere anche fatale.

Comunque, è innegabile che siamo ormai "assuefatti" all'influenza e al tasso di mortalità, anche se il lavoro da fare è ancora molto, considerando le basse coperture vaccinali e il controverso effetto sulla mortalità da parte degli antivirali a disposizione.
Ciò che ha innescato la reazione vigorosa da parte delle autorità sanitarie mondiali nei confronti di 2019-nCoV è innanzitutto **il carattere stesso di novità**: ci troviamo dinanzi a **un nuovo patogeno, che, per quanto in proporzione bassa rispetto a tanti altri tipi di virus, sta causando dei decessi in persone che viceversa non sarebbero morte**. Inoltre, non c'è né vaccino, né terapia.
Non vi è nemmeno parziale immunità nella popolazione, come accade per l'influenza, i cui virus rappresentano delle varianti di quelle circolate negli anni precedenti.
Infine, consideriamo un dato: **la letalità nei casi di Coronavirus notificati ad oggi* è del 3%.** È probabile, come affermano tutti gli esperti, che in realtà il denominatore su cui calcolarla sia molto più ampio, perché i casi accertati sono quelli dei pazienti che si ospedalizzano. **Ci saranno sicuramente molte persone in Cina con sintomi lievi che non si recano presso le strutture sanitarie**.
Ma cosa succederebbe se al mondo fossero colpite 200 milioni di persone? Significherebbe, stando a queste stime, avere sei milioni di morti, pressoché dieci volte il numero dei morti dell'influenza di stagione, che colpisce ogni anno circa un miliardo di persone secondo l'OMS.
Ecco perché fermare questo virus in tempo è così importante.
Benché non sia mortale alla stregua di altri patogeni in termini relativi, **colpendo un vasto numero di persone può determinare** in termini assoluti **un numero ragguardevole di**

***Nota: i dati sono aggiornati al 21 febbraio 2020**

decessi su scala globale che altrimenti non sarebbero occorsi.

Quali sono le differenze con la SARS?
Sono entrambi due coronavirus del genere beta. Sembrano condividere lo stesso target quale recettore per entrare nelle cellule bersaglio, ovvero l'enzima ACE2, ampiamente rappresentato nell'apparato respiratorio, specialmente nelle basse vie (trachea, bronchi e polmoni).
Possiamo affermare che le modalità di contagio e il periodo d'incubazione di Coronavirus e SARS siano sovrapponibili.
Tuttavia, balzano all'occhio **importanti differenze**: **il nuovo Coronavirus in poco più di un mese ha doppiato il numero di persone ufficialmente contagiate in circa due anni dal virus responsabile della SARS, risultando molto più diffusivo.** D'altro canto, anche la letalità è diversa, ma in questo caso a sfavore della SARS.

Si dice che la SARS fu peggiore di questa epidemia di Coronavirus, perché?
Il bilancio ufficiale della SARS fu di 8.098 infetti e 774 morti, ossia il 9,6% dei contagiati. Tra questi, purtroppo **anche Carlo Urbani**, unica vittima italiana, **infettivologo di straordinarie capacità** che contrasse il virus responsabile della SARS in Vietnam, ad Hanoi, ove lavorava per l'OMS. Grazie al suo grido d'allarme e al suo sacrificio i riflettori si accesero sull'epidemia di SARS, la cui **letalità fu dunque di un valore circa cinque volte superiore a quello desunto dalle cifre ufficiali per 2019-nCoV**.

Adesso che il virus è stato isolato, cosa si potrà fare in concreto e con quali tempistiche?
Bisogna fare una precisazione. Il virus è stato isolato in prima battuta da scienziati cinesi già il 10 gennaio, nello specifico da ricercatori dell'Università Fudan di Shangai, che hanno sequenziato in tempi rapidi l'intero genoma di circa 30.000 nucleotidi. In seguito, altri gruppi di ricerca in differenti paesi, in cui si sono diagnosticati casi di infezione da 2019-nCoV, hanno ottenuto lo stesso risultato. **In Europa, proprio poco prima dello Spallanzani, l'isolamento è avvenuto all'Istituto Pasteur a Parigi**. Ciò ovviamente non va a detrimento dell'eccellente lavoro svolto dal laboratorio di virologia dello Spallanzani: è chiaro che il virus si può isolare solo se si hanno materiali biologici, e secondo logica ciò è avvenuto in Cina che è il cuore dell'epidemia. Ad ogni modo, ottenere molteplici sequenziamenti del virus, da diversi pazienti in differenti posti del mondo, permette di ricostruire con precisione la sua **storia evolutiva e la sua capacità di mutazione**. Consente altresì di capire meglio quali sono i fattori che produce e che condizionano la virulenza. **Queste sono le premesse fondamentali per una terapia e per un vaccino**.
Circa, però, **le tempistiche**, si entra in un campo minato: dimostrare l'efficacia in laboratorio di un antivirale, dopo isolamento e coltivazione di un virus, non significa automaticamente che tale risultato sarà confermato nell'uomo, per tacere del profilo di sicurezza da vagliare con attenzione.
Anche per **i vaccini** il discorso è complesso: pur comprimendo il più possibile, fino a 6-12 mesi, la fase di preparazione del composto immunizzante, occorre testarne in vivo efficacia e sicurezza su campioni numerosi. **Non bisogna dunque dar credito a notizie miracolistiche sul timing di cure e vaccini, ma concentrarsi sull'infection control per bloccare la catena dei contagi.**

Questo articolo è stato pubblicato anche sul blog dell'autrice Medora il 7 febbraio https://studiomedora.it/coronavirus-facciamo-chiarezza/

Infezione da Coronavirus 2019-nCoV il mondo con il fiato sospeso

Alberto Enrico Maraolo

Epidemia o pandemia? Decisive le prossime settimane

Timeline degli eventi principali (aggiornata al 3 febbraio 2020)

Nell'ultimo giorno del 2019, il 31 dicembre, un primo report diramato dalla Commissione Sanitaria della municipalità di Wuhan ha annunciato l'esistenza di un cluster di 27 casi di polmonite atipica, presumibilmente virale, nella popolosa città della Cina centrale. Situata alla confluenza del Fiume Azzurro e del Fiume Han, Wuhan conta circa 11 milioni di abitanti ed è il capoluogo della provincia dello Hubei. Il giorno successivo, il mercato ittico locale, considerato l'epicentro dell'*outbreak*, è stato chiuso e sottoposto a decontaminazione.

L'8 gennaio 2020 le massime autorità sanitarie cinesi hanno dichiarato il nesso eziologico con un nuovo coronavirus, denominato ad oggi 2019-nCoV.
Il 10 gennaio il genoma del virus è stato svelato al mondo grazie al lavoro di un gruppo di ricerca dell'Università Fudan di Shangai. Lo stesso giorno è stato dichiarato il primo decesso ufficiale.
Il 13 gennaio le autorità sanitarie tailandesi hanno attestato il primo caso di infezione al di fuori della Cina: un soggetto di nazionalità cinese in viaggio da Wuhan.
Il 30 gennaio i primi casi di infezione da 2019-nCoV sono stati identificati in Italia, segnatamente una coppia di turisti cinesi sessantenni provenienti da Wuhan, ricoverati a Roma (dove avevano manifestato i sintomi) presso l'Istituto Lazzaro Spallanzani, centro di riferimento nazionale per le malattie infettive.
Sempre il 30 gennaio l'Organizzazione Mondiale della Sanità (OMS), tornando sui propri passi rispetto a una deliberazione diversa fatta la settimana precedente, ha dichiarato lo stato di emergenza globale.
Il 31 gennaio il governo italiano ha dichiarato lo stato di emergenza sanitaria per sei mesi, implementando una serie di misure straordinarie per potenziare i servizi sanitari territoriali e nosocomiali, nonché interrompendo i voli da e per la Cina.

Coronavirus: entità tutt'altro che sconosciute

I coronavirus sono virus a RNA divisi in quattro generi: alfa, beta, gamma e delta (gli ultimi due non rilevanti per l'uomo). Prendono il nome, che è un latinismo, dall'aspetto a "corona solare" delle loro particelle, in ragione della presenza di numerose spicole sulla superficie. Fino al secolo scorso i coronavirus sono stati considerati patogeni di scarsa rilevanza clinica, ma di grande importanza epidemiologica: i quattro tipi "umani" endemici a livello globale (HCoV 229E, NL63, OC43, KU1) sono responsabili infatti del 10-30% dei casi di raffreddore comune, e solo sporadicamente di infezioni più gravi: per esempio, in soggetti gravemente immunodepressi.

Lo scenario è cambiato con il nuovo secolo, in cui ogni decennio è stato caratterizzato dall'emergere di un nuovo coronavirus con diverso profilo di patogenicità e morbilità:

- nel 2002, nella provincia cinese del Guangdong, si registrò la comparsa di un coronavirus responsabile di un grave affezione respiratoria, la SARS (*severe acute respiratory syndrome*), internazionalmente riconosciuta solo nel 2013, che ha mietuto 774 vittime su un totale di 8098 casi accertati, specialmente nel Sud-Est asiatico (letalità 9,6%);
- nel 2012 il mondo ha fatto la conoscenza di un sesto coronavirus, associato alla MERS (*Middle East respiratory syndrome*), i cui dati ufficiali sono 858 morti su 2494 casi notificati, la maggioranza in Arabia Saudita (letalità 34,4%);
- nel gennaio 2020 è esploso l'*outbreak* in corso, legato a 2019-nCoV, nato già nelle ultime settimane del 2019.

In tutti e tre i casi si è configurato il quadro dello *spillover*, ovvero il salto di specie del virus da un *reservoir* (serbatoio) animale all'uomo, tramite un ospite intermedio. Nel caso di SARS e MERS, le specie serbatoio furono indentificate in alcuni tipi di pipistrello, mentre quelle che favorirono il passaggio all'uomo si sono rivelate essere lo zibetto e il dromedario, rispettivamente. Il virus responsabile della SARS sembra essere stato debellato, mentre casi rari di MERS si registrano ancora saltuariamente nella penisola arabica. Per quanto concerne 2019-nCoV, il *reservoir* è ignoto, ma si suppone che siano ancora una volta i pipistrelli, e sconosciuto è pure l'ospite intermedio: verosimilmente era presente nel mercato del pesce di Wuhan, ove non si vendevano solo prodotti ittici ma anche altri animali, spesso vivi e macellati sul posto come da tradizione locale. Tale promiscuità è uno dei fattori favorenti lo *spillover*. Conoscere *reservoir* e ospite intermedio è fondamentale, non solo a scopi scientifici, ma anche per il controllo dell'infezione: ogni prova tuttavia sembra essere stata distrutta con l'immediata chiusura del mercato di Wuhan.

Nuovo coronavirus: un po' di numeri, tra certezze e previsioni

Alla data del 3 febbraio, l'OMS riporta 17.391 casi confermati globalmente (in Cina, ove si registra il 99.1% delle infezioni, e in altri 23 paesi), con 362 morti (letalità pari al 2,1%). Nessun caso per ora è stato registrato in Africa e in America Latina.
Tuttavia, un recente studio di ricercatori di Hong Kong (Wu e collaboratori su Lancet), utilizzando raffinati modelli matematico-statistici, ha stimato alla data del 25 gennaio un numero di 75.815 infetti nella sola Wuhan (con una forbice da 37.304 a 130.330).

La forte discrepanza tra le stime e i casi notificati ha due spiegazioni, non mutuamente esclusive:
– le autorità cinesi stanno nascondendo la reale entità del problema;
– i casi accertati sono quelli in genere ospedalizzati, che rappresentano la punta di un iceberg molto più grande costituito da infezioni lievi o asintomatiche.

Per quanto riguarda la prima spiegazione, nonostante le ben nota censura cinese e i ritardi nell'identificare l'epidemia a dicembre, è evidente che il governo di Pechino non vuole ripetere l'esperienza della SARS, laddove omissioni e reticenze furono ancora più clamorose. Quindi, è verosimile che il numero di morti sia in realtà da rapportarsi a un denominatore molto più grande, fatto di numerosi casi lievi che passano inosservati e non sono diagnosticati per mancato accesso alle strutture ospedaliere.

Il periodo di incubazione dai primi report sembra variare dai 2 agli 11 giorni, ma, sulla scorta delle informazioni relative ai coronavirus precedenti, per precauzione si estende tale intervallo a 2 settimane.

Non è chiaro se la trasmissione, che avviene tramite *droplet*, le goccioline aero-trasmesse mediante colpi di tosse e/o starnuti, possa avere luogo anche da soggetti asintomatici o già nella fase di incubazione (l'influenza è per esempio contagiosa a partire dalle 24 ore prima della comparsa dei sintomi), come suggerito da alcune segnalazioni: i dati finora disponibili mostrano comunque che i soggetti sintomatici sono la maggiore causa della diffusione del contagio.
Un altro numero cruciale è il cosiddetto tasso di riproduzione netto, indicato come R_0, ovvero il numero di nuovi casi generati in media da un singolo soggetto durante il proprio periodo di contagiosità in una popolazione suscettibile: secondo le prime stime esso era circa 3,6-3,8. Tali valori, per quanto lontani da quelli di malattie molto contagiose quali il morbillo (oltre 10), implicavano il blocco di almeno il 72-75% delle trasmissioni tramite appropriate misure di *infection control* per interrompere il diffondersi dell'epidemia. Le ultime stime, come nello studio di Wu su Lancet, sono più basse, ossia valori di R_0 pari 2,6: ciò comunque implica la necessità di bloccare almeno il 60% delle trasmissioni per evitare il propagarsi dell'epidemia.

2019-nCoV: tirando le somme

L'infezione da 2019-nCoV nei casi sintomatici si presenta come un'affezione delle vie respiratorie di tipo simil-influenzale. Sintomi gastro-intestinali sono meno frequenti. Nei casi gravi si osservano polmonite, insufficienza respiratoria severa, insufficienza renale.
Nella più ampia casistica finora riportata (Li e colleghi sul New England Journal of Medicine), inerente a 425 pazienti da Wuhan, l'età media era di 59 anni, con una prevalenza di soggetti di sesso maschile (56%) e, nota importante, nessun caso al di sotto dei 15 anni. L'infezione è comunque possibile anche in età pediatrica, ma sembra essere meno rilevante clinicamente nelle prime età della vita. A maggior rischio di forme impegnative e potenzialmente fatali sono dunque anziani e soggetti con comorbilità di base.

Purtroppo, allo stato attuale non vi è né terapia eziologica né strategia preventiva mediante immunizzazione, trovandoci dinanzi a un nuovo patogeno. Ciò è verosimilmente alla base delle misure emergenziali intraprese, nonostante il tributo in termini di morti che ogni anno si paga nei confronti dell'influenza "classica" sia sempre considerevole (dai 290.000 ai 650.000 decessi su scala globale secondo le stime OMS), non scatenando tuttavia psicosi collettive.

È ancora presto per definire l'evoluzione dell'infezione da 2019-nCoV. In Cina si configura come un'epidemia su vasta scala e non è chiaro l'effetto della mega-quarantena imposta alla città di Wuhan, alla luce dei focolai scoppiati in numerose città cinesi. La trasmissione secondaria, in altri paesi, a partire da casi importati, è al momento estremamente modesta. Per escludere una pandemia occorrerà comunque un grande sforzo delle autorità cinesi, considerando che l'attuale "congelamento" di viaggi e attività commerciali deciso da molti paesi non potrà continuare all'infinito, alla luce del ruolo strategico della Cina nello scacchiere mondiale economico e politico.

Sicuramente è stata straordinaria la risposta della comunità scientifica e significativa anche quella delle principali riviste mediche mondiali, che hanno subito aperto spazi appositi nelle loro pagine web con pubblicazione in tempi rapidissimi di lavori fruibili in *open-access*. Numerosi anche gli articoli pubblicati in formato *pre-print* su *repository* on-line.

Bibliografia essenziale

- Gralinski E et al. Return of the Coronavirus: 2019-nCoV. Viruses 2020, 12, 135.
- Li Q et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. NEJM 2020. Last accessed 3 February 2020. https://www.nejm.org/doi/10.1056/NEJMoa2001316
- Rubin et al. Medical Journals and the 2019-nCoV Outbreak. NEJM 2020. Last accessed 3 February 2020. Last accessed 3 February 2020. https://www.nejm.org/doi/full/10.1056/NEJMe2001329
- Wu JT et al. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. The Lancet 2020. Last accessed 3 February 2020. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30260-9/fulltext
- WHO. Novel Coronavirus (2019-nCoV) situation reports- 14. Last accessed 3 February 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200203-sitrep-14-ncov.pdf?sfvrsn=f7347413_2

Coronavirus 2019-nCoV: dati incerti sui contagi in gravidanza

Enrico Finale

Il potenziale patogeno in gravidanza del nuovo coronavirus sembra essere simile a quello di SARS-CoV e di MERS-CoV

Primo caso di trasmissione verticale materno-fetale?

È di qualche giorno fa (02/02/2020) la notizia dei media di stato cinesi riguardante la diagnosi di infezione da nuovo Coronavirus 2019-nCoV in un neonato, nato a Wuhan, l'epicentro dell'epidemia, a 30 ore appena dalla nascita. Il neonato sarebbe il paziente più giovane a cui è stata diagnosticata l'infezione, ma la modalità di trasmissione non è stata ancora chiarita. Le due ipotesi principali sono:

- la trasmissione verticale (materno-fetale);
- il contagio intra-partum.

La mancanza di dati accurati non permette di postulare ipotesi precise. Tuttavia sembra chiaro che le infezioni respiratorie contratte

in gravidanza abbiano esiti peggiori in termini di mortalità, ricovero in terapia intensiva e morbilità rispetto alle infezioni nella popolazione non gravida. Questo dato è documentato, infatti, nell'epidemia di sindrome respiratoria acuta grave del 2002-2003 (SARS-CoV), durante la quale la mortalità delle donne gravide è risultata del 28% rispetto al 10% della popolazione non gravida. Nel corso di tale epidemia, non sono stati documentati casi di trasmissione materno-fetale.

Coronavirus e gravidanza: una storia che si ripete?

I coronavirus sono virus a RNA divisi in quattro generi: alfa, beta, gamma e delta (gli ultimi due non rilevanti per l'uomo). Prendono il nome, che è un latinismo, dall'aspetto a "corona solare" delle loro particelle, in ragione della presenza di numerose spicole sulla superficie. I due membri più famosi di questa famiglia sono i responsabili della sindrome respiratoria acuta grave (SARS-CoV) e della sindrome respiratoria del Medio Oriente (MERS-CoV). I dati in nostro possesso sono molto limitati, infatti durante l'epidemia di SARS del 2002-2003 sono stati rilevati 12 casi di infezione in gravidanza, mentre 11 sono state le donne che hanno contratto in gravidanza l'infezione da MERS-CoV. Gli esiti avversi sono stati: aborto spontaneo, restrizione o arresto della crescita fetale, parto pretermine e mortalità materna. Inoltre, per 6 su 11 neonati di mamme affette da MERS-CoV è stato necessario il ricovero in terapia intensiva neonatale e 3 di loro sono deceduti.

Quale condotta clinica per una sospetta infezione in gravidanza?

A oggi non sono disponibili dati che descrivano gli esiti di una infezione da 2019-nCoV in donne in gravidanza. Sembra però che il potenziale patogeno di 2019-nCoV sia simile a quello di SARS-CoV e di MERS-CoV, quindi il virus sarebbe capace di causare gravi esiti materni o perinatali. È raccomandato valutare attentamente qualsiasi malattia respiratoria febbrile in gravidanza operando opportune indagini diagnostiche. Inoltre, per le donne che hanno una storia di sintomi respiratori e/o febbrili entro 14 giorni dal viaggio di ritorno dalla regione di Wuhan in Cina, bisognerebbe prendere in considerazione il 2019-nCoV come una possibile causa.

Bibliografia

1. Wong SF, Chow KM, Leung TN et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. *Am J Obstet Gynecol.* 2004; 191: 292-297
2. Alfaraj SH, Al-Tawfiq JA, Memish ZA. Middle East respiratory syndrome coronavirus (MERS-CoV) infection during pregnancy: report of two cases & review of the literature. *J Microbiol Immunol Infect.* 2019; 52: 501-503
3. Mahase E. China coronavirus: what do we know so far?. *BMJ.* 2020; 368: m308
4. Favre G, Pomar L, Musso D et al. 2019-nCoV epidemic: what about pregnancies? *Lancet.* Corrispondence https://doi.org/10.1016/S0140-6736(20)30311-1
5. SOGC Infectious Disease Committee Statement on the Novel Coronavirus. The Society of Obstetricians and Gynaecologists of Canada: 2020

Diabete mellito, infezioni respiratorie e Sindrome COVID-19: evidenze disponibili e consigli pratici per la gestione delle persone con diabete durante il ricovero ospedaliero

Cristina Parrino

In Italia la sindrome nota come COVID-19 (*coronavirus disease* 2019) sta mettendo a dura prova il Sistema Sanitario Nazionale e ha generato un acceso dibattito a livello sociale, scientifico, mediatico, economico e politico.
La comunità scientifica sta progressivamente rendendo disponibili nuovi dati supportati dalle evidenze cliniche, ma sono **pochissime** le **certezze** riguardanti le **caratteristiche** e all'andamento **dell'infezione** da **SARS-COV-2** (*severe acute respiratory syndrome coronavirus 2*) nei pazienti con **diabete mellito**.

Le infezioni respiratorie nelle persone con diabete mellito e l'ospedalizzazione

L'influenza e le infezioni respiratorie sono malattie infettive comuni che si associano ad elevata mortalità e morbilità nelle persone in età avanzata e con patologie croniche [**1, 2**].
Nelle persone con **diabete mellito** è stata osservata una **predisposizione** a sviluppare alcune malattie infettive, tra cui le **infezioni acute delle basse vie respiratorie** causate da batteri [**3, 4**] e virus [**2**]. I meccanismi che determinano questa predisposizione non sono ancora del tutto noti, ma l'**iperglicemia** – responsabile di un'alterata funzione antibatterica dei neutrofili – e la presenza di **complicanze croniche diabete-correlate** sembrano avere un ruolo rilevante [**2, 5**]. Il sistema respiratorio delle persone con diabete potrebbe, infatti, essere interessato da **fenomeni microangiopatici** con conseguente riduzione degli scambi gassosi e della *compliance* polmonare. Sono riportati, inoltre, una maggiore suscettibilità a sviluppare infezioni delle basse vie respiratorie sostenute da **microrganismi atipici** ed episodi di **polmoniti di maggiore gravità** rispetto a chi non è affetto da diabete mellito [**6**].

Nelle ultime due decadi si sono verificate altre epidemie di infezioni respiratorie su scala globale tra cui l' **influenza A** (H1N1) nel 2009 e la **Middle East Respiratory Syndrome Coronavirus** (MERS-CoV) nel 2012 [**2**]. In entrambi i casi, il **diabete mellito** è stato individuato come **uno deifattori di rischio ospite-dipendente** ed era spesso presente nei soggetti che hanno sviluppato **complicanze fatali**[**2**]. Altre comorbidità, come l'ipertensione arteriosa, le patologie cardiache ischemiche, lo scompenso cardiaco, la malattia renale cronica allo stadio terminale sono state associate a maggiore letalità da MERS-CoV e la contemporanea presenza di due o tre comorbidità ha innalzato significativamente il tasso di letalità [**7**].
Quest'ultimo dato appare di particolare rilievo per la **popolazione con diabete mellito** a causa della frequente presenza di quadri con **pluri-comorbidità** (complicanze cardiovascolari e renali).
È noto che le persone con diabete mellito, rispetto a persone di pari età e dello stesso genere, hanno un **rischio aumentato di ricovero ospedaliero** per tutte le cause. Lo **scompenso cardiaco** e l'**insufficienza respiratoria** sono tra le prime cause di ricovero, rispettivamente nell'8 e nel 6% dei casi.

La gestione delle **malattie acute intercorrenti** e delle **infezioni** nelle persone con diabete mellito richiede particolare attenzione clinica, in quanto esse si accompagnano spesso ad un **peggioramento del compenso glicemico** a causa di:

- incremento della produzione epatica di glucosio;
- ridotta utilizzazione periferica del glucosio;
- aumento del fabbisogno insulinico;
- modifiche nell'alimentazione;
- riduzione dell'attività fisica.

Il ricovero in ospedale per malattie intercorrenti richiede, inoltre [**8**]:

- la **rivalutazione della terapia antidiabetica domiciliare**;
- l'individuazione di **obiettivi glicemici differenti**.

La Sindrome COVID-19 nei pazienti con diabete mellito: evidenze disponibili

Le evidenze attualmente disponibili sulle caratteristiche dell'infezione da **SARS-COV-2** nei pazienti con **diabete mellito** sono essenzialmente limitate al **numero di pazienti con infezione** confermata in ospedale, al **numero di pazienti trasferiti** nelle **unità di terapia intensiva** e al tasso di **letalità**. La **Tabella 1** riassume i dati dei principali studi retrospettivi condotti a Wuhan e nelle province di Hubei e Zhejiang. L'infezione sembra interessare maggiormente gli **uomini**, ad eccezione dello studio di Kui [12], ma dai dati disponibili non è possibile stabilire se questa differenza sia mantenuta anche nel gruppo delle persone con diabete mellito. La percentuale di pazienti con **infezione** da **SARS-COV-2** e **diabete mellito** varia nei vari studi tra il **2** e il **20%**. Negli studi non sono specificati il tipo di diabete (tipo 1 o tipo 2), il grado di compenso gliocometabolico, la terapia domiciliare praticata o le complicanze croniche associate.

Due studi riportano i dati di **accesso alle unità di terapia intensiva** (UTI) per i pazienti con diabete mellito: **8%** (1 su 13 trasferiti in UTI, *p value* 0.16) nello studio di Huang [14] e **22.2%** (8 su 36 trasferiti in UTI, *p value* 0.009) nello studio di Wang [9]. Lo studio di Xu [12], riporta che tra i 33 pazienti con **sintomatologia** di **durata superiore ai 10 giorni**

***Tabella 1**. Dati dei principali studi retrospettivi*

Studio	Casi (M/F)	Età media (anni)	Malattie Croniche n (%)	Diabete Mellito n (%)	MCeCV n(%)
Wang D [9]	138 (75/63)	56.0	64 (46.4%)	14 (10.1%)	20 (14.5%)
Kui [10]	137 (61/76)	57.0	27 (19.7%)	14, (10.2%)	10 (7.3%)
Chen [11]	99 (67/32)	55.5	50 (51%)	12 (12.1%)	50 (51%)
Xu [12]	62 (35/27)	41.0	20 (32%)	1 (2%)	1 (2%)
Yang [13]	52 (35/17)	59.7	21 (40%)	9 (17%)	12 (23.1%)
Huang [14]	41 (30/11)	49.0	13 (32%)	8 (20%)	6 (15%)

M: maschi, F: femmine; n: numero; %, percentuale; MC: malattie croniche; DM: diabete mellito; UTI: unità di terapia intensiva; MCeCV: malattie cerebro e cardiovascolari

dall'insorgenza della malattia il 39% presentava comorbidità e che, nello specifico, il **3%** era affetta da **diabete mellito**. Lo studio si Yang [13], condotto in pazienti in condizioni cliniche di criticità, ha riportato che il **22%** dei pazienti **non sopravvissuti** (7 su 32) e il **10%** dei pazienti **sopravvissuti** (2 su 20) all'infezione da **SARS-COV-2** era affetto da **diabete mellito**. Il report del Centre for Disease Control in Cina, che include 44672 casi di infezione confermata, riporta un **tasso di letalità** totale del 2.3%, del **7.3%** nelle persone con **diabete**, del 10.5% nelle persone con malattia cardiovascolare e del 49% nelle persone in condizioni cliniche di criticità [15].

Consigli pratici per il ricovero ospedaliero

Durante il ricovero ospedaliero per i pazienti con **diabete mellito noto** e **patologie acute**, si raccomanda [1, 8]:

- il **monitoraggio giornaliero della glicemia capillare** annotando i risultati in maniera chiara in cartella clinica o in un apposito foglio;
- il dosaggio di **glicemia a digiuno** ed **emoglobina glicosilata**, soprattutto se non eseguito nei 3 mesi precedenti;
- il raggiungimento di valori di **glicemia a digiuno <140 mg/dl** e **post-prandiali o random <180 mg/dl**;
- la **sospensione di farmaci antidiabetici orali o iniettivi diversi dall'insulina**;
- l'utilizzo di **terapia insulinica basale per via sottocutanea**, evitando di somministrare insulina solo al bisogno (*sliding scale*);
- l'eventuale **integrazione** con schema di correzione con **insulina rapida**
- la preparazione di un programma di trattamento dell'**ipoglicemia.**

Nei pazienti in **situazione critica** ricoverati in **terapia intensiva** si consiglia, invece, di:

- perseguire **target glicemici meno stringenti** (glicemia a digiuno 140-180 mg/dl)
- effettuare la **terapia insulinica in infusione venosa continua** seguendoalgoritmi basati sui frequenti controlli delle glicemie.

I pazienti con **primo riscontro di iperglicemia** in occasione di un ricovero, devono essere indirizzati a valutazione specialistica diabetologica [1].

Raccomandazioni per le persone con diabete mellito

Al momento non esistono specifiche indicazioni per le persone con diabete mellito riguardo l'infezione da SARS-COV-2. Come per la popolazione generale è consigliato di seguire le **raccomandazioni del Ministero della Salute** riportate anche sul sito dell'Associazione Medici Diabetologi (https://aemmedi.it/coronavirus-e-diabete/).

Conclusioni

I dati attualmente disponibili non permettono di eseguire analisi dettagliate sulle caratteristiche specifiche dell'infezione da SARS-COV-2 nelle persone con diabete mellito. Tuttavia, quanto osservato fino ad ora, sembra essere in linea con i precedenti riscontri in corso di epidemie di infezioni respiratorie.

Nei momenti di emergenza sanitaria è cruciale mantenere un approccio razionale ai problemi, al fine di fornire una guida ai pazienti e di poter mettere in atto strategie efficaci per le popolazioni esposte a maggior rischio.

Bibliografia

[1] Associazione Medici Diabetologi (AMD) – Società Italiana di Diabetologia (SID). Standard Italiani per la Cura del Diabete Mellito 2018. Disponibile in https://aemmedi.it/ e http://www.siditalia.it/. Ultima consultazione 04 Marzo 2020

[2] Alaa Badawi, Seung Gwan Ryoo Prevalence of diabetes in the 2009 influenza A (H1N1) and the Middle East respiratory syndrome coronavirus: a systematic review and meta-analysis Journal of Public Health Research 2016; volume 5:733

[3] Shah BR, Hux JE: Quantifying the risk of infectious diseases for people with diabetes. Diabetes Care 2003; 26: 510–513.

[4] Muller LM, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, Rutten GE: Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005; 41: 281–288.

[5] Knapp S. Diabetes and Infection: Is There a Link? – A Mini-Review Clinical Section / Mini-Review Gerontology 2013;59:99–104

[6] Ardigo D, Valtuena S., Zavaroni I, Baroni M.C., Delsignore R. Pulmonary complications in diabetes mellitus: the role of glycaemic control. Current Drug Targets – Inflammation & Allergy **Volume 3 , Issue 4 , 2004**

[7] Alqahtani FY, Aleanizy FS, Ali El Hadi Mohamed R, Alanazi MS, Mohamed N, Alrasheed MM, Abanmy N, Alhawassi T (2019). Prevalence of comorbidities in cases of Middle East respiratory syndrome coronavirus: a retrospective study. Epidemiology and Infection 147, e35, 1–5

[8] Sciacca L. La persona con diabete in ospedale – Il diabete in Italia a cura di Enzo Bonora e Giorgio Sesti. www.siditalia.it/pdf/Il Diabete in Italia_p.pdf Ultima consultazione 04 Marzo 2020

[9] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7

[10] Kui L, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, Xiao W, Wang YN, Zhong MH, Li CH, Li GC, Liu HG. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J (Engl). 2020 Feb 7

[11] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb 15;395(10223):507-513. Epub 2020 Jan 30

[12] Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Li SB, Wang HY, Zhang S, Gao HN, Sheng JF, Cai HL, Qiu YQ, Li LJ. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ. 2020 Feb 19;368:m606

[13] Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020 Feb 24. pii: S2213-2600(20)30079-5.

[14] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng

Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497-506. Epub 2020 Jan 24. Erratum in: Lancet. 2020 Jan 30

[**15**] Wu Z., McGoogan J., Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention JAMA Published online February 24, 2020

Occhi puntati al Coronavirus Congiuntivite virale da COVID-19

Paola Giorno

Dai dati elaborati in questi primi mesi di epidemia da COVID-19 la congiuntivite può essere uno dei primi sintomi presenti. Come tutte le congiuntiviti virali si caratterizza per presenza di intensa lacrimazione e rossore oculare.

Ma come dobbiamo comportarci, visti i recenti sviluppi dell'epidemia, durante la nostra pratica clinica?
Come da indicazione, già da fine gennaio da parte dell'American Academy of Ophthalmology e più recentemente anche dalla Società Oftalmologica Italiana, i pazienti con sospetta congiuntivite virale vanno isolati dagli altri pazienti, interrogati sulla eventuale presenza di altri sintomi concomitanti (febbre, tosse, gola infiammata, dolori muscolari, difficoltà respiratoria), interrogati sulla possibilità di essere transitati nelle aree infette o avere avuto contatti con pazienti infetti; i casi sospetti vanno eventualmente indirizzati ai presidi competenti e sottoposti al test per COVID-19.
I risultati dei test saranno poi disponibili entro 24h (Ospedale Sacco Milano, Ospedale Spallanzani Roma).
Per segnalare nuovi casi NON deve essere utilizzato il 118 ma i numeri da chiamare sono il 112 ed il 1500.
I medici oftalmologi, data la stretta vicinanza con il paziente alla lampada a fessura, dovranno utilizzare dispositivi di protezione (maschere, occhiali, guanti) e sebbene sia vero che la congiuntiva è una via di ingresso del virus nel nostro organismo, alcuni studi hanno ridimensionato la potenziale trasmissibilità attraverso la congiuntiva.

Il medico oculista deve informare il paziente di non toccare gli occhi con le mani, lavare spesso le mani, usare asciugami pulite giornalmente, cambiare spesso la fodera del cuscino, eliminare cosmetici oculari (matita per occhi, mascara, eyeliner, ombretti), non condividere cosmetici oculari con altre persone.
Sicuramente un ripasso sulle misure preventive recepite e divulgate dall'Istituto Superiore di Sanità (https://www.iss.it/?p=5108) e dal Consiglio dei Ministri (http://www.gover-

no.it/it/approfondimento/coronavirus-il-decreto-legge-23-febbraio-2020-e-il-dpcm-attuativo/14173) è molto utile per una migliore gestione dei pazienti critici.

Dall'autorevole rivista Science (Daniel Wrapp: Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation *Science* 19 Feb 2020:eabb2507 DOI: 10.1126/science.abb2507), intanto, arriva la pubblicazione della mappa 3D del coronavirus e, al momento, l'attenzione dei ricercatori è focalizzata sulla proteina SPIKE (protS) che sembrerebbe essere la proteina chiave per l'ingresso del coronavirus nelle membrane delle cellule; anche i primi test sul potenziale vaccino vanno avanti e tutta la comunità scientifica sta cooperando per avere tutte le informazioni possibili per attenuare e contrastare la diffusione dell'infezione.

COVID-19: quali indicazioni nell'attuale incertezza scientifica per salvaguardare la gravidanza, il parto e l'allattamento?

Arnulfo A[1], Capuano A[2], Perego B[2], Ponili A[2], Fiorentino R[2], Macchi C[2], Quintili F[2], Mazzoleni A[2], Bertolino M[2], Finale E[3]

[1]Direttore SOC Ostetricia e Ginecologia, ASL VCO, Verbania

[2]Dirigente Medico, SOC Ostetricia e Ginecologia, ASL VCO, Verbania

[3]Ostetrico Senior, SOC Ostetricia e Ginecologia, ASL VCO, Verbania

La mancanza di dati accurati e validati su COVID-19 (Corona Virus Disease) ha permesso di postulare sinora solo ipotesi dinamiche, che si aggiornano talvolta di giorno in giorno. Ad oggi cosa sappiamo e cosa dobbiamo garantire per proteggere la gravidanza, il parto e l'allattamento?

Quali indicazioni dare alle donne in gravidanza?

Sulla base di dati limitati ed esempi di casi di coronavirus precedenti (SARS-CoV e MERS-CoV) e un piccolo numero di casi COVID-19, si ritiene che le donne in gravidanza possano essere a maggior rischio rispetto alla popolazione generale per le infezioni respiratorie, infatti sono stati dimostrati esiti peggiori in termini di mortalità, ricovero in terapia intensiva e morbilità rispetto alle infezioni nella popolazione generale (1,2). L'incontrollata infodemia sul tema legato al SARS-CoV-2 (virus responsabile della COVID-19) ha generato indicazioni errate e talvolta allarmistiche. Le Ostetriche e i Ginecologi rappresentano le figure di riferimento nel percorso nascita delle donne italiane, e in quanto operatori privilegiati, sono chiamati alla diffusione di informazioni validate ed accreditate scientificamente. Non avendo allo stato attuale strumenti come un vaccino per contrastare COVID-19, per le donne in gravidanza e persone che le circondano è necessario far riferimento alle indicazioni del Ministero della Salute (3) che sono riconducibili alle comuni norme igieniche di prevenzione primaria: lavarsi spesso e accuratamente le mani,

evitare contatti con persone malate o che presentino sintomi riconducibili ad affezioni respiratorie ed evitare luoghi affollati. Inoltre, è opportuno sempre attenersi alle indicazioni ministeriali e delle istituzioni sanitarie regionali in caso di insorgenza di sintomi di malattie respiratorie.

Quali danni o eventi avversi fetali sono stati riscontrati nelle donne con COVID-19?

Le informazioni attualmente disponibili sulla comparsa di eventi avversi in gravidanza in donne infettate da COVID-19 sono scarse e basate su dati emersi da altre infezioni legate al coronavirus (SARS-CoV e MERS-CoV) durante la gravidanza (1,2,4). In questo contesto sono stati riportati casi di aborto spontaneo, di parto prematuro o di basso peso alla nascita. Inoltre, ricordiamo come lo sviluppo di febbre durante il primo trimestre di gravidanza possa aumentare il rischio di danni fetali.

Ad oggi non è chiaro se COVID-19 possa attraversare la via transplacentare causando una trasmissione verticale. Sono stati segnalati alcuni casi non comprovati di neonati che risultano positivi al virus poco dopo la nascita, ma sono necessari dati validati per capire come questi bambini sono stati infettati e se il virus può essere trasmesso o meno durante la gravidanza. La serie limitata di casi riportati in letteratura (4,5) non ha rilevato, in donne con sintomatologia clinica da COVID-19 in gravidanza, la presenza del virus nel liquido amniotico o nel sangue neonatale prelevato da cordone ombelicale.

Alcuni case reports riportano un parto prematuro in donne affette da COVID-19, ma non è chiaro se ciò sia legato all'infezione stessa o ad altri fattori coesistenti (4).

Quali raccomandazioni per l'assistenza ostetrica da diffondere tra gli operatori?

Il Centers for Disease Control and Prevention (CDC) ha emesso il documento *Interim Considerations for Infection Prevention and Control of Coronavirus Disease 2019 (COVID-19) in Inpatient Obstetric Healthcare Settings* (6)con lo scopo di offrire istruzioni organizzative alle strutture sanitarie che forniscono assistenza ostetrica alle donne in gravidanza con diagnosi confermata o sospetta di COVID-19, come i reparti di degenza, il triage ostetrico, blocco travaglio-parto ed in fine per la gestione della dimissione della diade mamma-bambino.

I punti salienti delle raccomandazioni sono:

- attivare una formazione di base e di aggiornamento per tutto il personale sanitario al fine di favorire la corretta aderenza alle pratiche di controllo delle infezioni e all'utilizzo dei dispositivi di protezione individuale (DPI);
- gli operatori sanitari devono informare tempestivamente il personale addetto al controllo delle infezioni presso la propria struttura in merito all'arrivo di una gestante con infezione confermata o sospetta di COVID-19;
- collocare la paziente con infezione confermata o sospetta di COVID-19 in isolamento, ove questo non fosse possibile, garantire il trasferimento in un'altra struttura sanitaria per garantire l'isolamento;
- offrire un percorso fisico e logistico dedicato per il travaglio-parto;
- i bambini nati da madri con COVID-19 confermato dovrebbero essere considerati come pazienti sospetti o in fase di accertamento diagnostico. Pertanto, i bambini devono essere isolati in base alle linee gui-

da per la prevenzione e il controllo delle infezioni per le persone in fase di indagine;
- per ridurre il rischio di trasmissione del virus che causa COVID-19 dalla madre al neonato, le strutture dovrebbero considerare di separare temporaneamente (stanze separate) la madre con diagnosi confermata o sospetta di COVID-19 sino al momento in cui è possibile sospendere ogni precauzione per la prevenzione della trasmissione;
- la dimissione per le donne ed i neonati dopo il parto dovrebbe seguire le raccomandazioni descritte nel documento *Interim Guidance for Discontinuation of Transmission-Based Precautions and Disposition of Hospitalized Patients with COVID-19* del CDC (7).

Quali modalità di parto per donne con sospetto o conferma di COVID-19?

Al momento, si sa molto poco su COVID-19, in particolare rispetto al suo effetto patogeno su donne in gravidanza e neonati (8). Come già detto precedentemente, i dati sono basati sugli esiti materno fetali in caso di altre infezioni legate al coronavirus (SARS-CoV e MERS-CoV) durante la gravidanza.
Allo stato attuale, in caso di condizioni materne stabili, non vi sono controindicazioni all'espletamento del parto per via vaginale. Non vi sono dati riguardo a differenze di trasmissione dell'infezione sulla base della modalità del parto e pertanto non vi indicazione elettiva al taglio cesareo nelle donne affette da infezione da COVID-19; rimangono valide le indicazioni attuali al taglio cesareo (9).

Quali modalità di alimentazione per i neonati di mamme con sospetto o conferma di COVID-19?

Gli attuali dati hanno dimostrato che Il SARS-CoV-2 non è stato rilevato nel latte materno. In considerazione degli indubbi benefici dell'allattamento e in base alle attuali raccomandazioni (10,11), l'allattamento materno deve essere avviato e/o mantenuto direttamente al seno o mediante latte spremuto manualmente o con metodi meccanici. Per ridurre il rischio di trasmissione, che avviene mediante droplet, le goccioline aero-trasmesse mediante colpi di tosse e/o starnuti, da madre a bambino, è raccomandata l'adozione di misure preventive come l'igiene delle mani prima di ogni poppata o di ogni spremitura del seno e l'uso della mascherina durante la poppata; in aggiunta alle raccomandazioni per i casi di separazione temporanea tra madre e bambino.

Bibliografia essenziale

1. Wong SF, Chow KM, Leung TN et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. Am J Obstet Gynecol. 2004; 191: 292-297
2. Alfaraj SH, Al-Tawfiq JA, Memish ZA. Middle East respiratory syndrome coronavirus (MERS-CoV) infection during pregnancy: report of two cases & review of the literature. J Microbiol Immunol Infect. 2019; 52: 501-503
3. Ministero della Salute. Nuovo coronavirus – Dieci comportamenti da seguire. http://www.salute.gov.it/portale/nuovocoronavirus/dettaglioOpuscoliNuovoCoronavirus.jsp?lingua=italiano&id=433 Ultimo accesso 4 Marzo 2020

4. Centers for Disease Control and Prevention (CDC) – Frequently Asked Questions and Answers: Coronavirus Disease 2019 (COVID-19) and Pregnancy. https://www.cdc.gov/coronavirus/2019-ncov/specific-groups/pregnancy-faq.html Ultimo accesso 5 Marzo
5. Chen H, Guo J, Wang C, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 2020;
6. Zhu H, Wang L, Fang C, et al. Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. *Transl Pediatr* 2020;9:51-60.
7. Centers for Disease Control and Prevention (CDC) – Interim Guidance for Discontinuation of Transmission-Based Precautions and Disposition of Hospitalized Patients with COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html Ultimo accesso 4 Marzo 2020
8. Centers for Disease Control and Prevention (CDC) – Interim Considerations for Infection Prevention and Control of Coronavirus Disease 2019 (COVID-19) in Inpatient Obstetric Healthcare Settings. https://www.cdc.gov/coronavirus/2019-ncov/hcp/inpatient-obstetric-healthcare-guidance.html Ultimo accesso 4 Marzo 2020
9. Favre G, Pomar L, Musso D, et al. 2019-nCoV epidemic: what about pregnancies? *Lancet* 2020;
10. Giusti A, Donati S, Silano M, D'Ancona FP. Istituto Superiore di Sanità, COVID-19: gravidanza, parto e allattamento. https://www.epicentro.iss.it/coronavirus/gravidanza-parto-allattamento Ultimo accesso 4 Marzo 2020
11. Centers for Disease Control and Prevention (CDC) – Interim Guidance on Breastfeeding for a Mother Confirmed or Under Investigation For COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/specific-groups/pregnancy-guidance-breastfeeding.html Ultimo accesso 4 Marzo 20202

Articoli full text open access di vari editori in inglese

In questa sezione troverete il full text di 10 articoli open access in inglese pubblicati su importanti riviste internazionali peer reviewed preceduti da un commento in italiano a cura del dottor Alberto E. Maraolo.

Interventi terapeutici per la SARS (il "vecchio" coronavirus): nessuna evidenza conclusiva

L'outbreak mondiale di SARS (*severe acute respiratory syndrome*) del 2002-2003 ha fatto conoscere al mondo il potenziale diffusivo del primo nuovo coronavirus del nuovo secolo. Nell'ottobre del 2003, l'Organizzazione Mondiale della Sanità istituì un apposito gruppo di studio che commissionò una revisione sistematica della letteratura inerenti ai protocolli di trattamento usati per l'infezione, screenando tutta la letteratura disponibile fino al febbraio 2005 in database quali MEDLINE, EMBASE, BIOSIS, CENTRAL.

La revisione ha incluso 54 studi clinici su pazienti affetti da SARS e 15 studi in vitro focalizzati sull'inibizione della replicazione del virus in laboratorio. Dagli studi in vitro è emersa la capacità inibente di ribavirina, lopinavir e interferone tipo I. La sintesi degli studi clinici tuttavia non ha evidenziato benefici su morbilità e mortalità associati a questi trattamenti né ad altri quali immunoglobuline e plasma di soggetti guariti.

Riferimento bibliografico

Stockman LJ et al. SARS: Systematic Review of Treatment Effect. PLOS Medicine 2006; 3: e343.

Segue articolo full text originale

OPEN ACCESS Freely available online

PLoS MEDICINE

SARS: Systematic Review of Treatment Effects

Lauren J. Stockman[1,2*], Richard Bellamy[3], Paul Garner[4]

1 Centers for Disease Control and Prevention, Respiratory and Enteric Viruses Branch, Atlanta, Georgia, United States of America, **2** Department of Veterans' Affairs, Atlanta Research and Education Foundation, Decatur, Georgia, United States of America, **3** James Cook University Hospital, Middlesbrough, United Kingdom, **4** Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Funding: This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Academic Editor: Donald Low, Mount Sinai Hospital, Canada

Citation: Stockman LJ, Bellamy R, Garner P (2006) SARS: Systematic review of treatment effects. PLoS Med 3(9): e343. DOI: 10.1371/journal.pmed.0030343

Received: January 5, 2006
Accepted: May 30, 2006
Published: September 12, 2006

DOI:
10.1371/journal.pmed.0030343

Abbreviations: ARDS, acute respiratory distress syndrome; IFN, interferon; IVIG, intravenous immunoglobulin; LPV/r, lopinavir and ritonavir; RCT, randomised controlled trial; SARS, severe acute respiratory syndrome; SARS-CoV, severe acute respiratory syndrome-associated coronavirus

* To whom correspondence should be addressed. E-mail: lstockman@cdc.gov

ABSTRACT

Background

The SARS outbreak of 2002–2003 presented clinicians with a new, life-threatening disease for which they had no experience in treating and no research on the effectiveness of treatment options. The World Health Organization (WHO) expert panel on SARS treatment requested a systematic review and comprehensive summary of treatments used for SARS-infected patients in order to guide future treatment and identify priorities for research.

Methods and Findings

In response to the WHO request we conducted a systematic review of the published literature on ribavirin, corticosteroids, lopinavir and ritonavir (LPV/r), type I interferon (IFN), intravenous immunoglobulin (IVIG), and SARS convalescent plasma from both in vitro studies and in SARS patients. We also searched for clinical trial evidence of treatment for acute respiratory distress syndrome. Sources of data were the literature databases MEDLINE, EMBASE, BIOSIS, and the Cochrane Central Register of Controlled Trials (CENTRAL) up to February 2005. Data from publications were extracted and evidence within studies was classified using predefined criteria. In total, 54 SARS treatment studies, 15 in vitro studies, and three acute respiratory distress syndrome studies met our inclusion criteria. Within in vitro studies, ribavirin, lopinavir, and type I IFN showed inhibition of SARS-CoV in tissue culture. In SARS-infected patient reports on ribavirin, 26 studies were classified as inconclusive, and four showed possible harm. Seven studies of convalescent plasma or IVIG, three of IFN type I, and two of LPV/r were inconclusive. In 29 studies of steroid use, 25 were inconclusive and four were classified as causing possible harm.

Conclusions

Despite an extensive literature reporting on SARS treatments, it was not possible to determine whether treatments benefited patients during the SARS outbreak. Some may have been harmful. Clinical trials should be designed to validate a standard protocol for dosage and timing, and to accrue data in real time during future outbreaks to monitor specific adverse effects and help inform treatment.

The Editors' Summary of this article follows the references.

Introduction

The severe acute respiratory syndrome (SARS) is a febrile respiratory illness primarily transmitted by respiratory droplets or close personal contact. A global outbreak of SARS between March 2003 and July 2003 caused over 8,000 probable or confirmed cases and 774 deaths [1]. The causative organism has been identified as a novel coronavirus (SARS-CoV) [2–4]. The overall mortality during the outbreak was estimated at 9.6% [5,6]. The overriding clinical feature of SARS is the rapidity with which many patients develop symptoms of acute respiratory distress syndrome (ARDS). This complication occurred in approximately 16% of all patients with SARS, and when it occurred was associated with a mortality rate of 50% [7,8].

At the time of the SARS epidemic it was not known what treatments would reduce SARS-related illness and deaths. Because the urgency of the international outbreak did not allow time for efficacy studies, physicians in Canada and Hong Kong treated the earliest patients with intravenous ribavirin, based on its broad-spectrum antiviral activity [9,10]. Corticosteroids and immune-modulating agents were often prescribed empirically. Soon after SARS-CoV was identified as the causative agent, antiviral screening programs were initiated; these programs reported several antiviral agents that inhibited SARS-CoV replication in vitro. These results led to the experimental use of protease inhibitors and interferon alpha (IFN-α) in the treatment of patients.

The most commonly used treatments for SARS are associated with adverse effects when used for other conditions (Table S1). In October 2003, the WHO established an International SARS Treatment Study Group, consisting of experts experienced in managing SARS. The group recommended a systematic review of potential treatment options to identify the targets for proper evaluation in trials should the disease recur [11]. This paper reports on this systematic review designed to summarise available evidence on the effects of ribavirin, lopinavir and ritonavir (LPV/r), corticosteroids, type I IFN, intravenous immunoglobulin (IVIG), or convalescent plasma in relation to (1) SARS-CoV replication inhibition in vitro; (2) mortality or morbidity in SARS patients; and (3) effects on ARDS in adult patients.

Methods

We prepared a protocol that defined our scope, inclusion criteria, and outcomes to be assessed. The interventions we included were defined by the WHO: ribavirin, LPV/r, corticosteroids, type I IFN, convalescent plasma, or IVIG.

The types of study we included were: (1) in vitro studies, in which the authors examined inhibition of SARS-CoV viral replication, *and* data from an assay in human or animal cell line; (2) in vivo studies, which included randomised controlled trial (RCT), *or* prospective uncontrolled study design, *or* retrospective cohort design, *or* case-control design, *or* a case series, *and* patients treated for SARS, *and* ten or more patients; and (3) studies of ARDS that included RCT, *or* systematic review, *and* treatment for ARDS or acute lung injury, *and* 20 or more patients. In February 2005, we systematically searched the literature databases MEDLINE, EMBASE, BIOSIS, and the Cochrane Central Register of Controlled Trials (CENTRAL) for articles that included the selected treatments (Table S2).

The full text of each identified study was retrieved and each was independently reviewed by two authors (LS and RB). Publications in Chinese were selected after review of the English abstract. Unpublished data were not sought, as the task of summarising existing published data was extensive and the International SARS Treatment Group indicated that much of the clinical data had already been published. We used the QUOROM checklist to help ensure the quality of this review (Table S3).

Data from the full text of studies in English were extracted independently by two authors (LS and RB). Data from the Chinese literature were extracted with the assistance of a translator. Because the Chinese articles were reviewed by only one author, the consistency of the translated information with that from English articles was maintained by subsequent discussion with the translator to verify the extracted data.

We established explicit criteria to assess the level of evidence for each human treatment study (Box 1). Since the treatments chosen for evaluation were often given in combination, evidence was classified by the treatment that was given to all patients in the cohort or given to some with the author's intention of studying its effects. If putative effects within a study included several drugs, then we extracted data for each intervention. The level of evidence was independently classified by two authors (LS and RB). Chinese studies were appraised and classified in the same way using translated information extracted from each report. Discrepancies were resolved by consensus.

Results

In vitro evidence was available in 15 studies. Clinical evidence of SARS treatment in humans was reported in 54 studies (37 in English, 17 in Chinese). Three studies addressed treatment of ARDS (Figure 1).

Ribavirin

In vitro. We found six studies that described the antiviral effect of ribavirin in vitro (Table S4); four showed an antiviral effect (Table S5). A synergistic antiviral effect between ribavirin and type I IFN (IFN-β1a or leukocytic IFN-α) was described in two studies performed in human cell lines and Vero cell lines [12,13].

In SARS patients. We found 24 studies that described ribavirin treatment in cohorts larger than ten patients (Table S6). Our formal assessment classified 20 studies as "inconclusive," due to study design or because the effect of ribavirin could not be distinguished from the effects of other treatments (such as steroids and antiviral drugs). Four publications presented evidence of possible harm (14–17). Three of these studies, each of which included over 100 patients, documented a fall in haemoglobin levels after ribavirin treatment when compared to levels in patients before treatment [14–16]. Of patients treated with ribavirin, 49/138 to 67/110 (36%–61%) developed haemolytic anaemia, a recognised complication with this drug, although it is not possible to rule out the possibility that SARS-CoV infection caused the haemolytic anaemia, as there is no control group. One study noted that over 29% of SARS patients had some degree of liver dysfunction indicated by ALT levels higher than normal,

Box 1. Categories of Evidence Defined for In Vivo Studies of Treatments in SARS Patients

"*Inconclusive*" if a study could not be used to inform a decision about treatment efficacy due to having either outcomes which were not reported consistently, an inconsistent treatment regimen, no control group or a control group which was a likely source of bias. A control group was considered a likely source of bias if there were differences in co-morbidities, sex, age and markers of severe disease compared to the treatment group.

"*Possible harm*" if a study reported adverse effects of treatment that were consistent with adverse effects reported with the use of the drug in the treatment of other conditions. Evidence of direct causality was not required. A study could be classified as suggesting possible harm from the drug even if the study had methodological weaknesses.

"*Possible benefit*" if a study had evidence of benefit for an important outcome measure which was recorded consistently (e.g., case fatality, need for mechanical ventilation, duration of hospitalization, frequency of ARDS) in patients treated in a defined way compared to a valid control group. A control group was considered valid if randomized, or if patient characteristics and illness severity were comparable to the treatment group. Evidence of direct causality was not required.

"*Definite harm*" if a study contained statistically significant evidence of harm demonstrated in a double-blind randomized trial, which did not contain serious methodological weaknesses.

"*Definite benefit*" if a study contained statistically significant evidence of harm demonstrated in a double-blind randomized trial, which did not contain serious methodological weaknesses.

and the number of patients with this complication increased to over 75% after ribavirin treatment (Table S7) [17].

In the Chinese literature six additional reports described patients with SARS treated with ribavirin (often with steroids). These six reports were determined to be inconclusive in the evaluation of treatment for SARS (Tables S8 and S9).

LPV/r

In vitro. Of three studies, two demonstrated that lopinavir inhibits cytopathic effects of SARS-CoV in fetal rhesus monkey kidney cells (Table S4). One study showed detectable but reduced activity in Vero-E6 cells [13], and one study concluded that neither lopinavir nor ritonavir had an effect [18]. A synergistic effect of lopinavir with ribavirin has been reported (Table S5).

In SARS patients. We found two studies of LPV/r (lopinavir 400 mg with ritonavir 100 mg orally every 12 h) in cohorts larger than ten patients (Table S6). Patients also received ribavirin and corticosteroids. LPV/r use was compared among three groups of patients: those who received it as an early SARS treatment, those who received it as a late treatment, and those who did not receive it at all.

When LPV/r was added as an initial treatment to ribavirin and corticosteroid therapy, the death rate was lower than among those who received ribavirin and corticosteroids (1/44 [2.3%] versus 99/634 [15.6%]; $p < 0.05$) [19]. A second study of this regimen reported fewer episodes of ARDS or death compared with historical controls who had not received LPV/r (1/41 [2.4%] versus 32/111 [28.8%]; $p < 0.001$) (Table S7) [20]. Both studies were determined to be inconclusive due to possible bias in the selection of control group or treatment allocation.

No additional studies were identified from the Chinese literature.

Corticosteroids

In vitro. No studies were found on the cytopathic effect of corticosteroids alone against SARS-CoV. Corticosteroids act as immunomodulatory agents, and therefore studies to measure direct antiviral effects in vitro were not expected.

In SARS patients. Fifteen articles examined corticosteroid treatment in ten or more patients. Of these cohorts 13 were also treated with ribavirin (Table S6). We determined that 13 of the 15 studies were inconclusive. Of these, in an uncontrolled and nonrandomised study, 95/107 (89%) of patients treated with high-dose methylprednisolone (0.5–1 mg/kg prednisolone on day 3 of illness, followed by hydrocortisolone 100 mg every 8 h, and pulse-doses of methylprednisolone 0.5 g IV for 3 d) after the first week of illness recovered from progressive lung disease (Table S7) [16].

Two studies contained evidence of possible harm from corticosteroids [21,22]. One measured SARS-CoV plasma viral load across time after fever onset in a randomized, double-blind, placebo-controlled trial; corticosteroid use within the first week of illness was associated with delayed viral clearance. The other study, which was case-controlled, found that patients with psychosis received higher cumulative doses of steroids than patients without psychosis (10,975 mg versus 6,780 mg; $p = 0.017$) [22].

In the Chinese literature, we found 14 reports in which steroids were used (Table S8 and Table S9). Twelve studies were inconclusive and two showed possible harm. One study reported diabetes onset associated with methylprednisolone treatment [23]. Another study (an uncontrolled, retrospective study of 40 SARS patients) reported avascular necrosis and osteoporosis among corticosteroid-treated SARS patients [24].

In ARDS patients. Three clinical trials examined the effect of corticosteroids on mortality in patients with established ARDS (Table S10). In two trials, high-dose methylprednisolone given for approximately 2 d was not effective for early ARDS [25,26]. One small RCT that used a regimen of lower dose methylprednisolone (2 mg/kg per day), tapered after 2 wk, showed possible evidence of ARDS improvement (Table S11) [27].

IFN Type I

In vitro. Twelve in vitro studies with data on the antiviral effect of IFN type I have been reported, and all demonstrated an antiviral effect against SARS-CoV (six for IFN-α and ten for IFN-β) (Tables S4 and S5). Antiviral effects have been demonstrated in monkey (Vero; Vero-E6), fetal rhesus monkey kidney (fRhK-4), and human (Caco2, CL14, and HPEK) cell lines.

Three reports presented evidence that IFN-β was superior against SARS-CoV compared to IFN-α and found rIFN-α2 virtually ineffective against SARS-CoV compared to other IFNs [28]. Synergistic effects were reported for leukocytic IFN-α with ribavirin [13], IFN-β with ribavirin [12,13] and IFN-β with IFN-γ [28,29].

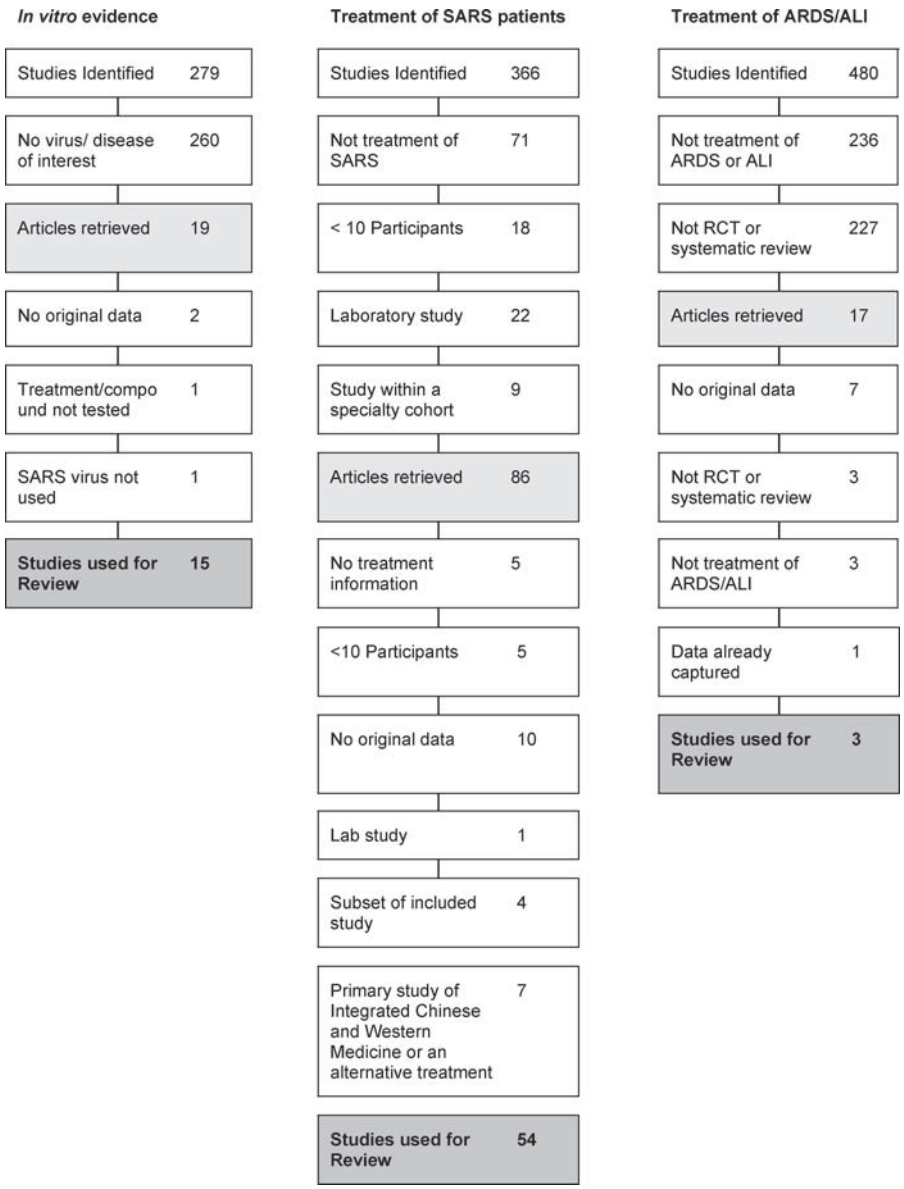


Figure 1. Process of Study Exclusion for Each Objective Category
DOI: 10.1371/journal.pmed.0030343.g001

In SARS patients. Two studies of IFN-α given with steroids and/or ribavirin were reported (Table S6). No significant difference was seen in outcome between IFN-α treatment group and those treated with other regimens. Results of both studies were inconclusive due to a lack of a consistent treatment regimen or suitable control group (Table S7).

In the Chinese literature, one additional study reported the use of IFN-α as part of a regimen that included ribavirin and steroids [30]. We determined this study to be inconclusive because a variety of treatments given masked the effect of IFN-α alone (Table S8 and Table S9).

Convalescent Plasma or Immunoglobulin

In vitro. No studies were found on the cytopathic effect of this treatment on SARS-CoV. Convalescent plasma and IVIG act as immunomodulatory agents and therefore studies to measure direct antiviral effects in vitro were not expected.

In SARS patients. Five studies of either IVIG or convalescent plasma treatment given in addition to steroids and ribavirin were reported for treatment of SARS (Table S6). These studies were inconclusive, because the effect of convalescent plasma or IVIG could not be discerned from effects of patient comorbidities, stage of illness, or effect of other treatments (Table S7).

In the Chinese literature, two additional studies reported evidence on the effect of convalescent plasma as a treatment for SARS [30,31]. These studies were inconclusive (Table S8 and Table S9).

Evidence collected on the benefit or harm of drugs used to treat SARS is summarized in Table 1.

Discussion

The rapid spread and subsequent control of SARS precluded controlled clinical treatment trials during the

Table 1. Summary of the Evidence for Benefit or Harm of Drugs Used to Treat SARS

Treatment	Inconclusive[a]	Possible Harm[a]	Total Studies with Evidence (English and Chinese)[b]
Ribavirin	26	4	30
Corticosteroid	25	4	29
LPV/r	2	0	2
IFN-α	3	0	3
Convalescent plasma or Immunoglobulin	7	0	7

[a]Studies were classified into six categories, but there were four categories without any studies: "possible benefit," "possible harm," "definite benefit," "definite harm" (see Box 1).
[b]Studies totalled 54; some reported on more than one drug.
DOI: 10.1371/journal.pmed.0030343.t001

outbreak of 2002–2003. In this report we summarize the results of a systematic evaluation of the findings from published reports of treatments used for SARS during the epidemic. Publications from the Chinese literature were included to capture as much evidence as possible. We developed specific criteria (Box 1) to look for large, obvious effects of benefit, adverse or poor outcomes, or evidence of potential benefit that could be used to prioritise future research of SARS treatments. A summary of this evidence in SARS patients is shown in Table 1.

Despite thirty reports of SARS-infected patients treated with ribavirin, there is no convincing evidence that it led to recovery. Haemolytic anaemia, a recognized side effect of this treatment, was observed in three studies. We would infer from these findings that any future use of ribavirin for SARS should be within the context of a controlled trial with close attention given to adverse effects.

Corticosteroids were commonly prescribed to SARS patients with worsening pulmonary disease or progressing abnormalities on chest X-rays. Treatment regimens varied widely but can be classified into two groups, early treatment and rescue treatment given at a later stage of illness. It is difficult to make a clear recommendation about whether corticosteroids should be used to treat SARS-associated lung injury in any stage of illness, particularly as the drug is immunosuppressive and may delay viral clearance if given before viral replication is controlled [21]. Of added concern are infectious complications, avascular necrosis, and steroid-induced psychosis—recognized adverse effects of corticosteroid use. Fungal superinfection and aspergillosis have been noted in case reports and autopsy findings of SARS patients given corticosteroids at high doses or for prolonged periods [32,33]. This review has found evidence of avascular necrosis and steroid-induced psychosis in SARS patients.

Seven studies of treatment with convalescent plasma or IVIG, three with IFN type I, and two with LPV/r were inconclusive by the criteria used in our analyses. Authors of four of the IVIG studies commented that patients seemed to improve upon treatment, but that more controlled trials of this approach are needed to provide evidence of an effect for SARS.

Important caveats should be considered in this review. Most of the studies of SARS patients were descriptions of the natural course of the disease and had not been designed to reliably assess the effects of the treatments used. Patient characteristics such as age and presence of diabetes mellitus have been associated with severe disease and can confound treatment effects. A diagnostic test for early SARS illness was not validated or widely available, and in general, treatment was initiated once patients fulfilled a clinical and epidemiological case definition. It is possible that the inclusion of patients without laboratory confirmation of SARS-CoV infection in this review could cause an underestimate of any true effect of antiviral treatment on SARS.

The variation in treatment regimens—particularly the wide range in doses, duration of therapy, and route of administration of ribavirin and corticosteroids—is a major obstacle to a clear interpretation of the data in this review. The nonstandardised collection of clinical information limits the conclusions that can be drawn from a retrospective analysis. We suggest that, in the event of a future outbreak of SARS-CoV or another novel agent, attempts be made to develop treatment protocols and to collect and contribute information for a standardized minimum dataset that could facilitate analysis of treatment outcomes among different settings. As observational studies pose problems of interpretation, the need is great for good-quality randomised trials, despite the difficulties in organising such trials.

Supporting Information

Table S1. Rationale for Treatments and Recognized Adverse Effects
Found at DOI: 10.1371/journal.pmed.0030343.st001 (55 KB DOC).

Table S2. Method of Systematic Review
(A) Search strategy, step 1: Select the treatments.
(B) Search strategy, step 2: Narrow the scope.
(C) Inclusion criteria and information sought from each study.
Found at DOI: 10.1371/journal.pmed.0030343.st002 (45 KB DOC).

Table S3. QUOROM Statement
Found at DOI: 10.1371/journal.pmed.0030343.st003 (50 KB DOC).

Table S4. Description of SARS-CoV Replication Studies: Assay Type and Outcomes Measured
Found at DOI: 10.1371/journal.pmed.0030343.st004 (79 KB DOC).

Table S5. Results from SARS-CoV Replication Studies: Inhibition of SARS-CoV Replication
Found at DOI: 10.1371/journal.pmed.0030343.st005 (84 KB DOC).

Table S6. Description of Studies within SARS Patients
Found at DOI: 10.1371/journal.pmed.0030343.st006 (186 KB DOC).

Table S7. Results of Treatment within SARS Patients (English literature)
Found at DOI: 10.1371/journal.pmed.0030343.st007 (183 KB DOC).

Table S8. Description of Studies of SARS Patients (Chinese Literature)
Found at DOI: 10.1371/journal.pmed.0030343.st008 (108 KB DOC).

Table S9. Results of Treatment within SARS Patients (Chinese Literature)
Found at DOI: 10.1371/journal.pmed.0030343.st009 (93 KB DOC).

Table S10. Description of Studies of ARDS or ALI
Found at DOI: 10.1371/journal.pmed.0030343.st010 (50 KB DOC).

Table S11. Results of Treatment of ARDS or ALI
Found at DOI: 10.1371/journal.pmed.0030343.st011 (48 KB DOC).

Acknowledgments

We acknowledge Dr. Larry J. Anderson for his review of manuscript drafts and consultation on in vitro data for SARS treatment, Dr. HaoQiang Zheng for extracting and translating data from the articles published in Chinese, Ms. Vittoria Lutje for helping with the literature search strategy, and the WHO International SARS Treatment Study Group for prioritising this research project and identifying treatments for review.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Author contributions. LS, RB, and PG drafted the protocol for this review. LS and RB reviewed all abstracts and extracted data. All authors appraised included studies, interpreted results, commented critically on the manuscript, and contributed text to the final version.

References

1. World Health Organization (2004) Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. Available: http://www.who.int/csr/sars/country/table2004_04_21/en/index.html. Accessed 26 July 2005.
2. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, et al. (2003) A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 348: 1953–1966.
3. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, et al. (2003) Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361: 1319–1325.
4. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, et al. (2003) Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348: 1967–1976.
5. Peiris JS, Yuen KY, Osterhaus AD, Stohr K. (2003) The severe acute respiratory syndrome. N Engl J Med 2003: 2431–2441.
6. Leung GM, Hedley AJ, Ho LM, Chau P, Wong IO, et al. (2004) The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: An analysis of all 1755 patients. Ann Intern Med 141: 662–673.
7. Fowler RA, Lapinsky SE, Hallett D, Detsky AS, Sibbald WJ, et al. (2003) Critically ill patients with severe acute respiratory syndrome. JAMA 290: 367–373.
8. Lew TW, Kwek TK, Tai D, Earnest A, Loo S, et al. Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. JAMA 290: 374–380.
9. Poutanen SM, Low DE, Henry B, Finkelstein S, Rose D, et al. (2003) Identification of severe acute respiratory syndrome in Canada. N Engl J Med 348: 1995–2005.
10. Lee N, Hui D, Wu A, Chan P, Cameron P, et al. (2003) A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 348: 1986–1994.
11. World Health Organization (2003) Future clinical trials for SARS, Informal meeting and workshop. Draft Report. Geneva: World Health Organization.
12. Morgenstern B, Michaelis M, Baer PC, Doerr HW, Cinatl J Jr. (2005) Ribavirin and interferon-beta synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines. Biochem Biophys Res Commun 326: 905–908.
13. Chen F, Chan KH, Jiang Y, Kao RY, Lu HT, et al. (2004) In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. J Clin Virol 31: 69–75.
14. Knowles SR, Phillips EJ, Dresser L, Matukas L (2003) Common adverse events associated with the use of ribavirin for severe acute respiratory syndrome in Canada. Clin Infect Dis 37: 1139–1142.
15. Booth CM, Matukas LM, Tomlinson GA, Rachlis AR, Rose DB, et al. (2003) Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. JAMA 289: 2801–2809.
16. Sung JJ, Wu A, Joynt GM, Yuen KY, Lee N, et al. (2004) Severe acute respiratory syndrome: Report of treatment and outcome after a major outbreak. Thorax 59: 414–420.
17. Wong WM, Ho JC, Hung IF, Ng W, Lam YM, et al. (2003) Temporal patterns of hepatic dysfunction and disease severity in patients with SARS (4). JAMA 290: 2663–2665.
18. Yamamoto N, Yang R, Yoshinaka Y, Amari S, Nakano T, et al. (2004) HIV protease inhibitor nelfinavir inhibits replication of SARS-associated coronavirus. Biochem Biophys Res Commun 318: 719–725.
19. Chan KS, Lai ST, Chu CM, Tsui E, Tam CY, et al. (2003) Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: A multicentre retrospective matched cohort study. Hong Kong Med J 9: 399–406.
20. Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, et al. (2004). Role of lopinavir/ritonavir in the treatment of SARS: Initial virological and clinical findings. Thorax 59: 252–256.
21. Lee N, Allen Chan KC, Hui DS, Ng EK, Wu A, et al. (2004) Effects of early corticosteroid treatment on plasma SARS-associated coronavirus RNA concentrations in adult patients. J Clin Virol 31: 304–309.
22. Lee DTS, Wing YK, Leung HCM, Sung JJY, Ng YK, et al. (2004) Factors associated with psychosis among patients with severe acute respiratory syndrome: A case-control study. Clin Infect Dis 39: 1247–1249.
23. Xiao JZ, Ma L, Gao J, Yang ZJ, Xing XY, et al. (2004) [Glucocorticoid-induced diabetes in severe acute respiratory syndrome: The impact of high dosage and duration of methylprednisolone therapy]. Zhonghua Nei Ke Za Zhi 43: 179–182.
24. Li YM, Wang SX, Gao HS, Wang JG, Wei CS, et al. (2004) [Factors of avascular necrosis of femoral head and osteoporosis in SARS patients' convalescence]. Zhonghua Yi Xue Za Zhi 84: 1348–1353.
25. Bernard GR, Luce JM, Sprung CL, Rinaldo JE, Tate RM, et al. (1987) High-dose corticosteroids in patients with the adult respiratory distress syndrome. N Engl J Med 317: 1565–1570.
26. Weigelt JA, Norcross JF, Borman KR, Snyder WH 3rd (1985) Early steroid therapy for respiratory failure. Arch Surg 120: 536–540.
27. Meduri GU, Headley AS, Golden E, Carson SJ, Umberger RA, et al. (1998) Effect of prolonged methylprednisolone therapy in unresolving acute respiratory distress syndrome: A randomized controlled trial. JAMA 280: 159–165.
28. Scagnolari C, Vicenzi E, Bellomi F, Stillitano MG, Pinna D, Poli G, et al. (2004) Increased sensitivity of SARS-coronavirus to a combination of human type I and type II interferons. Antivir Ther 9: 1003–1011.
29. Sainz B Jr, Mossel EC, Peters CJ, Garry RF (2004) Interferon-beta and interferon-gamma synergistically inhibit the replication of severe acute respiratory syndrome-associated coronavirus (SARS-CoV). Virology 329: 11–17.
30. Wu W, Wang JF, Liu PM, Chen WX, Yin SM, et al. (2003) [Clinical features of 96 patients with severe acute respiratory syndrome from a hospital outbreak]. Zhonghua Nei Ke Za Zhi 42: 453–457.
31. Li ZZ, Shen KL, Wei XM, Wang HL, Lu J, et al. (2003) [Clinical analysis of pediatric SARS cases in Beijing]. Zhonghua Er Ke Za Zhi 41: 574–577.
32. Hwang DM, Chamberlain DW, Poutanen SM, Low DE, Asa SL, et al. (2005) Pulmonary pathology of severe acute respiratory syndrome in Toronto. Mod Pathol 18: 1–10.
33. Wang H, Ding Y, Li X, Yang L, Zhang W, et al. (2003) Fatal aspergillosis in a patient with SARS who was treated with corticosteroids. N Engl J Med 349: 507–508.

Editors' Summary

Background. Severe acute respiratory syndrome (SARS) is caused by a virus; the main symptoms are pneumonia and fever. The virus is usually passed on when people sneeze or cough. SARS became a much-talked about disease in 2003, when over 8,000 cases and 774 deaths occurred worldwide. The situation was alarming, because the first-ever cases had only just appeared in 2002, in China, so the best way to treat this new disease was unknown. Not many drugs are effective against viruses, and all doctors can usually do with a viral disease is to treat specific symptoms (e.g., fever and inflammation) and rely on the body's own immune system to fight off the virus itself. However, in recent years a number of antiviral drugs have been developed (for example, several are in use against HIV/AIDS), so there was hope that some of them might be active against SARS. Steroids were also often used in SARS treatment to try to reduce the inflammation of the lungs. In order to find out which, if any, of the potential treatments for SARS were effective, a number of research studies were carried out, both during and since the recent outbreak.

Why Was This Study Done? Health care decisions should be based on all the information that is available. It is important to try to bring together all the reliable evidence that exists on each possible treatment for a disease. The process of doing so is called a systematic review. In October 2003 the World Health Organization (WHO) established an International SARS Treatment Study Group, consisting of experts experienced in treating patients with SARS. The group recommended a systematic review of potential treatments for SARS. In particular, it was considered important to summarise the available evidence on the use of certain antiviral drugs (ribavirin, lopinavir, and ritonavir), steroids, and proteins called immunoglobulins, which are found naturally in human blood. The WHO group wanted to know how these treatments affected the virus outside the body ("in vitro") and whether it helped the condition of patients and reduced the death rate, particularly in those patients who developed the dangerous complication called acute respiratory distress syndrome (ARDS). This study is a systematic review conducted in response to the WHO request.

What Did the Researchers Do and Find? They did no new work with patients or in the laboratory. Instead they conducted a comprehensive search of the scientific and medical literature for published studies that fitted their carefully predefined selection criteria. They found 54 SARS treatment studies, 15 in vitro studies, and three ARDS studies that met these criteria. Some of the in vitro studies with the antiviral drugs found that a particular drug reduced the reproduction rate of the viruses, but most of the studies of these drugs in patients were inconclusive. Of 29 studies on steroid use, 25 were inconclusive and four found that the treatment caused possible harm.

What Do These Findings Mean? From the published studies, it is not possible to say whether any of the treatments used against SARS were effective. No cases of SARS have been reported since 2004 but it is always possible that the same or a similar virus might cause outbreaks in the future. It is disappointing that none of the research on SARS is likely to be useful in helping to decide on the best treatments to use in such an outbreak. The authors discuss the weaknesses of the studies they found and urge that more effective methods of research be applied, in a timely fashion, in any similar outbreaks in the future. While the systematic review suggests that we do not know which if any of the potential treatments against SARS are effective, its recommendations mean that researchers should at least be better prepared to learn from potential future outbreaks.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0030343.

- Wikipedia entry on SARS (Wikipedia is a free online encyclopedia that anyone can edit)
- MedlinePlus pages on SARS
- Wikipedia entry on systematic reviews, which includes links to other Web sites where more detailed information may be found

Il controllo delle malattie infettive diffusive in Cina prima di COVID-19: cenni storici

La Cina, quale paese più popoloso al mondo, ha rappresentato una sfida non da poco per il controllo delle malattie trasmissibili negli ultimi decenni. Gli avvenimenti politici hanno ovviamente influenzato l'organizzazione sanitaria: dopo la II guerra mondiale e la guerra civile che portò alla costituzione della Repubblica Popolare Cinese nel 1949, per circa trent'anni si è assistito a un notevole miglioramento di vari indicatori di salute pubblica, quali l'aspettativa di vita e la mortalità infantile, il tutto accompagnato da risultati quali l'eradicazione del vaiolo.

Le riforme che hanno trasformato, a partire dagli anni '80, la Cina in un'economia di mercato, seppur sotto rigido controllo statale, hanno però indebolito la sanità territoriale, specialmente nelle aree rurali. Programmi di natura top-down hanno ottenuto risultati contrastanti. Le difficoltà poi nell'affrontare l'epidemia di SARS (*severe acute respiratory syndrome*) sono state la cartina di tornasole delle contraddizioni interne a una potenza globale.

Riferimento bibliografico

Hipgrave LJ. Communicable disease control in China: From Mao to now. J Glob Health 2011; 1: 224-38.

Segue articolo full text originale

journal of global health

PAPERS

Communicable disease control in China: From Mao to now

David Hipgrave*

Nossal Institute for Global Health, University of Melbourne, Melbourne, Australia

* Formerly UNICEF China Chief of Health, Nutrition, and Water and Environmental Sanitation

China's progress on communicable disease control (CDC) in the 30 years after establishment of the People's Republic in 1949 is widely regarded as remarkable. Life expectancy soared by around 30 years, infant mortality plummeted and smallpox, sexually transmitted diseases and many other infections were either eliminated or decreased massively in incidence, largely as a result of CDC. By the mid-1970s, China was already undergoing the epidemiologic transition, years ahead of other nations of similar economic status. These early successes can be attributed initially to population mobilization, mass campaigns and a focus on sanitation, hygiene, clean water and clean delivery, and occurred despite political instability and slow economic progress. The 10-year Cultural Revolution from 1966 brought many hardships, but also clinical care and continuing public health programs to the masses through community-funded medical schemes and the establishment of community-based health workers. These people-focused approaches broke down with China's market reforms from 1980. Village doctors turned to private practice as community funding ceased, and the attention paid to rural public health declined. CDC relied on vertical programs, some of them successful (such as elimination of lymphatic filariasis and child immunisation), but others (such as control of schistosomiasis and tuberculosis) demonstrating only intermittent progress due to failed strategies or reliance on support by the poorest governments and health workers, who could not or would not collaborate. In addition, China's laissez-faire approach to public health placed it at great risk, as evidenced by the outbreak in 2003 of the Severe Acute Respiratory Syndrome. Since then, major changes to disease reporting, the priority given to CDC including through major new domestic resources and reform of China's health system offer encouragement for CDC. While decentralized funding and varying quality diagnosis, reporting and treatment of infectious diseases remain major challenges, national priority on CDC in China is high.

Correspondence to:
David Hipgrave
Box 1049
Brighton Rd RPA
Elwood, 3184
Australia
dhipgrave@gmail.com

There are two things about modern China with which most readers will be familiar. The first is that it is the world's most populous nation: recently released census data revealed that China's population in 2010 approached 1.34 billion. This is below the figure of 1.4 billion anticipated,

as the growth rate of 0.57% per annum has fallen substantially. China's population, along with that in the rest of the world, began to grow very rapidly from the mid-18th century, from an estimated 177 million in 1750 to approximately 430 million in 1850 and 580 million by 1950 (1). The low annual growth rate of only 0.3% during the century to 1950 changed with the relative political stability since 1949; the population sky-rocketed in the 1950s and 1960s. This resulted in public advocacy on family planning ("later, longer, fewer") and finally the one-child policy that has applied to around two-thirds of couples since the late 1970s (2). The need for population control in China was based not only on the formerly high fecundity of Chinese women, but also the rapid fall in the crude death rate that accompanied the establishment of the People's Republic of China (PRC). This fall was largely due to communicable disease control (CDC).

The second familiar aspect is China's meteoric economic development, with an average annual growth rate of around 10% over the last 30 years. China's economic performance is now a major influence on global financial markets, with the developed world now heavily dependent on China's continued growth. Less familiar is the fact that this stellar economic performance only commenced in the second half of the 62 years since 1949.

Both of these familiar aspects of China almost certainly depend heavily on the fact that China's population, for the most part, became relatively healthy compared to residents in nations at a similar stage of development during the first 30 years of the PRC, and certainly much healthier that it was in 1949. By 1980, life expectancy in low-income China (67 years) exceeded that of most nations of similar gross domestic product per capita by seven years (as estimated in 1984), and indeed exceeded that of many middle-income nations (3). Although with some exceptions the health of China's population depends now largely on control of non-communicable diseases (NCDs), the foundation of China's population health, particularly the amazing growth in life expentancy from an estimated 32 years in 1949, depended almost entirely on CDC.

This paper provides an overview of CDC in China since the defeat of China's Nationalists by Mao Zedong's Communists. With regular reference to the contemporary political and economic context, it first describes what is known about disease epidemiology and causes of death before 1949, the strategies used in CDC and the major achievements made in the next 30 years. It follows with a description of the decline of CDC and community-funded public health in the context of China's economic reform, the vertical and vertically-funded disease-control programs and, through SARS, the awakening in China of the risk posed to the people and the nation of ignoring disease surveillance and a population-level approach to public health. The paper finishes with an overview of the status of certain communicable diseases and CDC in China in 2011, and analysis of the impact of China's current health system reforms on this issue.

1949–1979: COMMUNICABLE DISEASE CONTROL AND MORTALITY REDUCTION ON A MASS SCALE

When the Communists founded the PRC in early October, 1949, they established control of one of the most impoverished nations on earth. After a century of domination by Europeans, the fall of the Qing Empire was followed by partial Japanese occupation and a 38 year civil war. The vast majority of the population were engaged in subsistence agriculture, and a survey on the causes of death conducted in 1929–31 revealed that more than half of all deaths were caused by infectious diseases. A list of leading health problems before 1949 (**Table 1**) is noteworthy for the virtual absence of non-communicable diseases (King and Locke, 1983; as cited in ref. 1), and rural health care was in very poor supply (4–6).

Early disease-control programs

The political turmoil and slow socioeconomic development in China between 1949 and 1978 obscure its impressive progress in population health during those years. The Communists were quick to make good on promises of land-reform and establishment of a national "people's" government. In 1950 a Marriage Law was enacted, providing equal rights for women, and the first National Health Congress established a focus on rural health, disease prevention through campaigns, and collaboration between western and traditional Chinese medicine. The focus on improving rural health and on CDC persisted until the 1980s.

Early efforts in public health included work on vaccination, environmental sanitation and hygiene (including the early introduction of composting of night-soil to reduce the concentration of intestinal parasites) and the development of organized CDC programs. Incredibly, between 1950 and 1952, over 512 million of China's ~600 million people were vaccinated against smallpox, massively reducing case numbers; the last outbreak of smallpox in China occurred in 1960, 20 years before global eradication (7). By 1957, more than two-thirds of China's then ~2050 counties had an epidemic prevention station (EPS) or more specialized centres for the control of specific diseases (such as malaria, plague, schistosomiasis, leishmaniasis and brucellosis) modelled on those established in the Soviet Union earlier in the 20th century. Their efforts included "patriotic health campaigns" focusing on ensuring

Table 1 Major health problems in China before 1949*

INFECTIONS			OTHER CONDITIONS
Amoebic dysentery	Japanese B encephalitis	Schistosomiasis	Bronchitis
Ancylostomiasis (hookworm)	Leishmaniasis	Smallpox	Diabetes
Anthrax	Leprosy	Syphilis	Encephalomyelitis
Ascariasis (roundworm)	Leptospirosis	Taeniasis	Fluorosis
Bacillary dysentery	Malaria	Tahyna fever/encephalitis	Kashin-Beck disease
Brucellosis	Measles	Tapeworm	Glaucoma
Cholera	Mumps	Tetanus	Goiter
Clonorchiasis (liver fluke)	Paragonimiasis	Tick-borne relapsing fever	Keshan disease
Dengue fever	Pertussis	Trachoma	Malnutrition
Diphtheria	Plague	Tuberculosis	Nephritis
Enterobiasis (pinworm)	Pneumonia	Typhoid/paratyphoid	Opium addiction
Epidemic meningitis	Polio	Typhus	Rickets
Fasciolopsiasis	Rabies	Varicella	
Filariasis	Rheumatic fever	Viral haemorrhagic fever	
Gonorrhoea	Ringworm	Viral hepatitis	
Influenza	Scarlet fever		

*Data adapted from Banister, 1987 (1).

a clean environment and safe drinking water, vector control, latrine construction and human waste disposal. Each of these short-term interventions (on average twice a year, lasting for around a week) required the mass mobilization of peasants, and so served to increase the "health literacy" of the rural population (1,6–8).

Apart from targeted vaccination, other nascent disease control programs emerged. As a result, cases of typhus dropped by 95% in the 1950s, and there were also major attempts to control gonorrhoea and syphilis (considered by the communists to be social diseases associated with liberal western attitudes and affecting up to 50% of some population groups), first with imported and then domestically produced penicillin. Prostitution was also outlawed and the status of women elevated (6,9–11). Vaccination and campaigns against diphtheria and tuberculosis (TB) also commenced in the 1950s. In the late 1950s, another campaign to "exterminate the four pests" (sparrows, rats, flies and mosquitoes) was avidly implemented, albeit with major negative results when the exploding locust population decimated crop harvests, contributing to famine from 1958–1960 (1,7).

Newborn and puerperal infection rates also decreased tremendously during this period, with the re-training of up to 750 000 traditional midwives and establishment of 2380 maternal and child health (MCH) centres by 1952. No other type of medical facility increased at this rate, and a major result was the decline in neonatal tetanus, down from up to 5% of all newborns to a fraction of this figure (1,6).

Whilst tremendously successful, these mostly preventive care efforts, however, do not infer that rural Chinese had access to clinical care in the 1950s. Patriotic health campaigns were highly effective in CDC but were rarely sustained for more than a month; diseases not addressed by the campaigns were simply neglected and curative care was virtually unavailable outside the cities. Medical schools primarily trained doctors for hospital work. Rural Chinese basically only had access to Chinese herbal medicine and other traditional healers until well into the 1960s (1,6).

In addition, the patriotic health campaigns occurred in the context of major political instability in China. After liberation of the masses in 1949 and a period of relative self-control by peasants of their newly acquired land and produce, Mao introduced a set of disastrous social and economic policies involving community and agricultural collectivization. Motivated by jealousy of the Soviet Union and the west and his perspectives that the rural masses should be both self-sufficient and the source of grain for the cities, Mao promoted the Great Leap Forward from 1958–1960. This included new cultivation methods that failed dismally, further reducing the harvest. Impacted also by adverse weather and the locusts, the resulting famine resulted in the death by starvation of tens of millions, temporarily halting the rapid population growth wrought by successes in CDC.

Village doctors bring curative care, knowledge and a public health approach to the masses

After the disastrous Great Leap period, Mao retreated into the political background and China entered a period of relative political quiet in the early 1960s. Collectivization was relaxed and the patriotic health campaigns continued. EPSs grew in number, reaching around 2500 by 1965 (7), and vertical CDC programs expanded. With a return to food security (albeit with rationing), population growth resumed and life expectancy continued to grow (1). However, unhappy with his perception that the revolution was faltering, development was slowing and that his own political star was fading, in 1966 Mao launched the Cultural Revolution, throwing China into a ten-year period of political and economic chaos. The Revolution was characterized by mass mobilization of urban youth against author-

ity, closure of higher education institutions and a "return to the countryside" policy to pursue revolution as an abstract concept (6).

One positive element of this period, however, was the establishment of a village level cooperative medical scheme (CMS) managed by "barefoot doctors", a new cadre of community-level health worker who brought basic curative care, health education and a continuous rather than campaign-style public health approach to rural peasants (12). Later hailed as the foundation of primary health care (13), China's barefoot doctors rose in number from around one million in 1970 to a peak of around 1.8 million in 1977. Many barefoot doctors were selected from, functioned in the context of and were largely funded by local production brigades (roughly 1000–2000 people in a geographic area) or teams (200–400 people). These brigades had replaced the failed, larger communes established during the Great Leap years, and apart from their commitment to providing grain to the national coffers at fixed prices, were semi-autonomous. Other barefoot doctors were selected from among the urban youths who were "sent down" to the countryside, ill-equipped to farm but educated and literate enough to be trained in basic health care. As a result, and also because each brigade had variable financial capacity to fund its CMS, the quality of health care provided by the barefoot doctors (and an even more basic cadre of community health worker, the health aide, whose numbers added an additional 3.7 million to the community health workforce in 1970) varied widely (**Figure 1**). It also depended on the level and quality of training (which varied from one to six months in duration) and supervision. Some villages also benefited from physicians who had been sent down from the cities for ideological re-education but continued to provide health care, and also from oversight by the EPS team at county level (6,12,14).

The roles of the barefoot doctors and health aides included environmental sanitation, health education, disease screening, surveillance and control, basic clinical care or referral and family planning. CDC continued to benefit from management of water sources and disposal of human excreta (including through composting), improvements in wells, toilets, stables, cooking areas and the local environment, and specific disease control programs through reducing stagnant water, spraying and other measures to control flies, fleas and mosquitoes. Although the barefoot doctors continued the "prevention first" approach to CDC established in the 1950s under the guidance of the Patriotic Health Campaign Coordination Office (a quasi-Ministerial agency only absorbed into the Ministry of Health in 1989), clinical links were established via a three-tier referral network from village through commune to county levels, with supervision in the reverse direction. This three-tier network persists today (7,15,16).

Although politically inseparable from the prevailing harsh limitations on personal expression and movement (6), CDC in China in the late 1960s and throughout the 1970s thus benefited from a large cohort of community-level staff (health aides, barefoot doctors, sent-down physicians and also midwives) with a basic knowledge of health and hygiene (14). These cadres continued the "serve the people" philosophy of the patriotic campaigns initiated in the 1950s, but with a bottom-up rather than top-down approach (4) and, along with other determinants, especially education, contributed in a highly cost-effective way to the continually plummeting crude death and child mortality rates, rising life expectancy and to CDC in rural China.

Perspectives on the origin of China's village doctors. The rationale for the introduction of the barefoot doctors, and their impact, has interested recent scholars, and the different perspectives are summarized in **Figure 2**. One thesis holds that they were part of Mao's goal of improving the level of literacy in China, itself the antithesis of the contemporary philosophy that education was bourgeois (17). In support of this theory is the observation that improvements

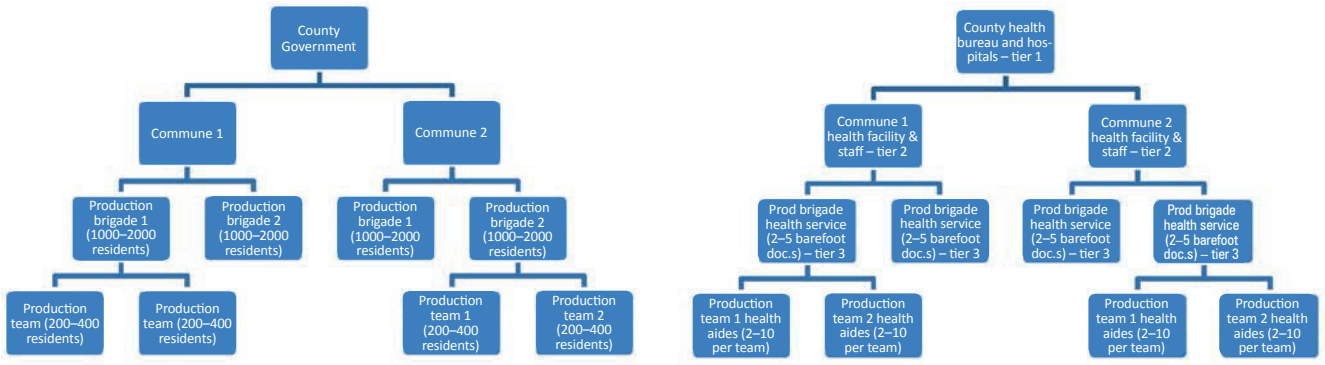


Figure 1 The rural government and health system in 1960s–1970s China, depicting the three-tier network.

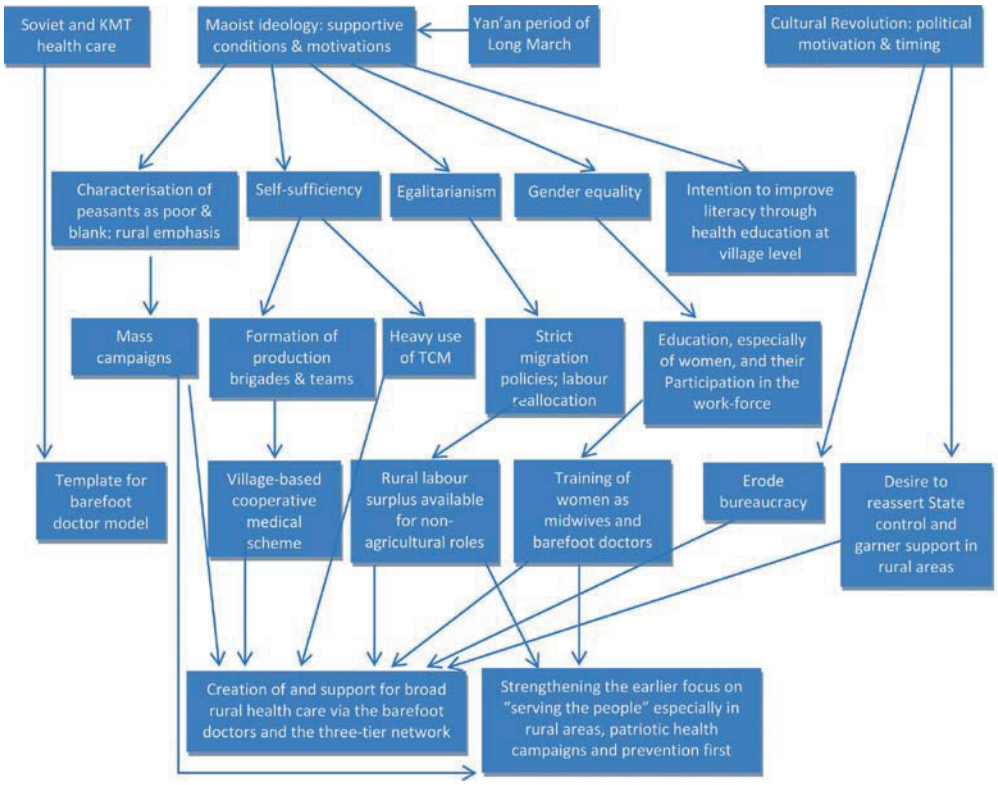


Figure 2 Origins and determinants of China's barefoot doctors program (after Bien, 2008; ref. 6).

in education complemented the public health campaigns in reducing mortality (8). Another points to three influences: (i) models provided by Guomindang experiments on basic primary health care in the 1930s and 1940s, and the Soviet 'feldshers' (field doctors who provided primary health care at village level, supervised by trained staff at higher levels); (ii) the ideology of self-sufficiency, gender equality and egalitarianism (with the peasants as the agents, not just the beneficiaries of revolution), taken up by the Mao and the Communists in Yan'an in the 1940s (also giving rise to the preference for the traditional Chinese medicine practiced by barefoot doctors) and (iii) the political situation in the mid-1960s, which gave rise to Mao's contention that the urban elite (including the Ministry of Health) was ignoring the backbone of the Revolution, the rural peasantry (18), and undermining his reliance on them for his own status. Having failed at commune level during the Great Leap years, self-sufficiency was instead introduced at the more stable village or brigade level, represented in the health sector by the barefoot doctors and the CMS. Whilst benefiting the health status of the population, the benefit for the nation as a whole through collectivization at this lower level was the resulting reliable supply of grain for the cities (6).

Another feature of this period that facilitated the success of the barefoot doctor movement was the surfeit of labour generated by the burgeoning population, movement restrictions that kept the rural population above 80% of the total until 1979 and the relocation of educated urban dwellers to the countryside. Sent-down physicians and urban-educated barefoot doctors made the most of the relative physical ease and prestige of their work, and the fact that income was somewhat less dependent on state-controlled grain prices (6,14).

Finally, the focus on gender equity was another significant influence on the success of the barefoot doctor movement. Although only one third of officially designated barefoot doctors were female, women made up the majority of midwives and health aides, who also functioned as barefoot doctors and contributed to CDC. Ideologies promoting female participation in the rural labour force provided the barefoot doctors program with a significant source of labour, also contributing to effective MCH programs (6).

Along with various social determinants, particularly education and the emancipation of women, the outcome of the PRC's efforts in CDC and community-funded public health during its first 30 years are remarkable indeed, considering its relatively poor economic progress. A 1984 World Bank report suggests China was already entering the epidemiologic transition in the mid-1970s, with deaths due to communicable disease down to only 25%, compared to 44% in other low income countries and virtually all deaths be-

fore 1949 (3). Other reports document an increase in life expectancy from 35 to 68 years, a fall in the crude mortality rate of around 66% and infant mortality from around 250 to 40 deaths per 1000 live births and a decrease in malaria prevalence from 5.5 to 0.3% of the population, between 1949 and 1981 (7,14).

MARKETISATION AND THE BREAKDOWN OF COMMUNITY-FUNDED PRIMARY HEALTH CARE IN THE 1980S

The introduction of market reforms in 1980 heralded the collapse of China's brigade system, the CMS and the funding for the barefoot doctors (19), many of whom abandoned this work in favour of farming (which became more profitable with the abandonment of collective agriculture), or moved to the cities in the context of relaxed movement control) (20). From 1979 to 1984, CMS coverage fell from 80–90% of peasants to 40–45%, and those schemes remaining offered variable and limited coverage (14). By 1986, rural CMS coverage had fallen to 9.5% (15). The number of the newly-named "village doctors" fell to around 1.2 million by 1984, and their supervision and regular retraining also decreased dramatically (14,21), resulting in falling standards despite them handling almost 50% of the nation's clinical work. Having lost their income from the CMS, village doctors have ever since relied on generation of income from fees and the sale of drugs, resulting in abandonment of public health work and major problems with over-prescribing of drugs and inappropriate use of parenteral preparations (20–25), problems that are only now being addressed (26). Payment for health care became the responsibility of the individual; government spending on health as recently as 2008 averaged less than 1% of the national budget (27) and the plummeting affordability of health care resulted in persistently low rates of rural hospital bed occupancy (15,28) and slower declines in infant mortality and the crude death rate (7,29,30). Urban-rural disparities in health funding, facility quality, staff allocations and service uptake rose dramatically, demonstrating burgeoning inequity in China's health sector (15,21,29). Financial decentralization was applied in both the commercial and public sectors, leaving province and county governments to mostly fend for themselves, with minimal support from the national government (14); government funding as a proportion of total health expenditure fell from almost 40% in the early 1980s to below 20% by 1993 and has remained below this figure until 2007 (21,31). It has risen sharply in recent years. Another source has the government's share of total health expenditure falling from 32% to 16% from 1978 to 2002 (32).

Public health in general and CDC in particular suffered badly in this new marketised milieu, as funding for preventive health services declined and the government adopted a laissez-faire attitude to preventive health (19,33). While overall government health resources increased at an annual rate of 6% from 1980 to 1995, the rate of increase for public health services was only 4.8%. The public health share of the health budget declined from 15–18% in the 1970s to 10.6% by 1995. Hospitals were the winners, as the focus on prevention switched to treatment (19). While the falls in county level public health funding were bad, they were worse at commune (now called township) level, with funds covering less than 60% of salaries and nothing else by 1993 (34). Funding of preventive health activities at village level that characterized the barefoot doctor period totally disappeared over the 1980s, and is only now beginning to recover with China's current health system reforms. One reason for this numeric increase but relative decline in public health funding was the increasing number of public health staff and facilities. As with curative services, government successfully reduced the cost but maintained the operation of public health services and CDC by encouraging self-sufficiency through the charging of fees for inspections and vertical programs, and there is good evidence of reduced wastage and improved productivity and efficiency in this regard (34). However, again there were problems with over-servicing of facilities who could afford the fees and ignoring weaker ones with greater problems. In food safety, this was shown by the rising incidence of hepatitis, typhoid and paratyphoid from 1979 to 1988 (19). Public preventive health activities (public goods without direct benefit to consumers) that were not profitable were often neglected or ignored; fees were even charged for vertical disease control programs (such as those against TB and schistosomiasis) despite national targets indicating their priority in the 7th and 8th five-year plans (7), an acknowledgement of the reliance on their implementation by staff whose participation could only be guaranteed with a financial incentive (or who charged fees regardless of services being notionally free). New charges for specific activities such as vaccination, control of schistosomiasis, TB, leprosy and also MCH reduced their uptake and impact. However, rather than cancel vaccination fees, the government introduced an immunization insurance scheme to counter falling coverage (apparently with good effect) (15), and fees for routine vaccination were only officially banned in 2007; the sale of optional vaccines (including several of the new vaccines recommended by WHO) remains a significant source of income for CDCs in China. Decentralisation of social service funding resulted in differential services according to counties' and townships' ability to fund them and the level of prioritization of public health by local authorities. Vertical lines of communication and control of the health system by health authorities also weakened (19). Administration of township health services gradually devolved from county to township governments, and the

township health facilities divided into clinical and preventive sections, with separate funding, revenue and reporting streams (15). Most EPSs reported to local government rather than to higher levels within the health hierarchy, exacerbating the politicization of data and probability of its desensitization. Local government was usually more concerned with economic than social indicators, and disinclined to report bad news like disease outbreaks. They were also disinclined to spend public money on CDC when they could use it to make the county rich.

In this context, the Ministry of Health had a limited role in initiating and sustaining public health programs. The 1989 Law on Control of Infectious Diseases and associated regulations conferred authority and responsibility to act on local governments, the EPSs, specialized institutes and hospitals (7), but these were weakly implemented. Despite encouraging descriptions of a computerized national disease reporting system and surveillance points and associated auditing (35,36) (**Figure 3**), and the piloting of a model CDC centre in Shanghai from 1998 (37), China did not commence modernization of its public health services until 2002 (38), when the old, mainly academic Chinese Academy of Preventive Medicine and county and provincial EPS network was replaced by a revitalized network of Centres for Disease Control modelled on those in the United States and dedicated to public health. There was no compulsory notifiable disease reporting system until 2004. **Figure 3** depicts the disease reporting system that applied from 1985 to 2003, the years of marketisation and the collapse of coordinated CDC. The system was characterized by poor enforcement and weak oversight; annual reports showed that some health providers and hospitals did not bother to report data. During the early weeks of the Severe Acute Respiratory Syndrome (SARS) in 2003, the multiple treating facilities were either not reporting cases, or were reporting to multiple different and non-coordinated authorities (39).

VERTICAL DISEASE CONTROL PROGRAMS REPLACE COMMUNITY APPROACHES TO CDC IN THE 1990S

As indicated, enormous progress was made on CDC in China in the first 30 years of the PRC, so even ignoring the economic reforms it is perhaps not surprising that the approach to CDC changed dramatically after 1980. In the new environment, abstract problems such as those with hygiene and sanitation that caused common, usually non-fatal diseases like diarrhoea and hepatitis now attracted less attention. Indeed, hygiene and sanitation are good examples of public goods whose priority lagged during this period, and China's progress on the safe water and sanitation

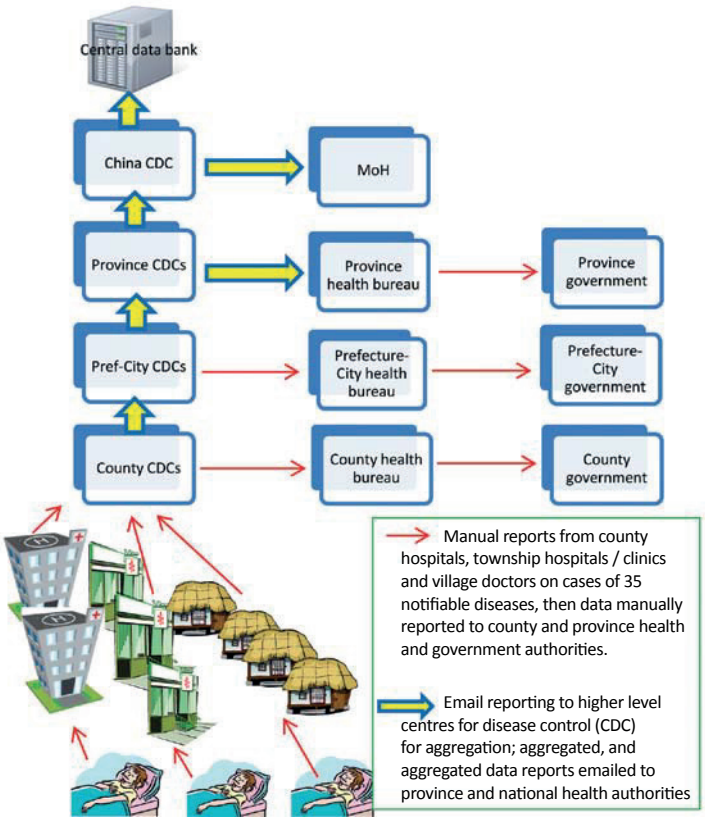


Figure 3 Notifiable disease reporting in China, 1985–2003.

indicators for the seventh Millennium Development Goal has been relatively slow (40).

In this new context, the former campaign approach to CDC was replaced by longer term vertical programs, and several related successes in China are documented even during this period when CDC in China was generally marketised. These include elimination of lymphatic filariasis using diethylcarbamazine-citrate fortified cooking salt (41), marked reductions in malaria and control of poliomyelitis (local transmission of which in China was eliminated from 1996 until 2011) and other vaccine-preventable diseases (7,42,43). For the most part, these successes resulted from disease-specific programs, such as the Expanded Program on Immunisation (EPI) and various other long-term projects. A description of two of these priority disease control programs in the context of CDC in China follows.

Various approaches to the control of schistosomiasis

Schistosomiasis control has been prioritized in China since the 1950s, with various strategies involving coordination between public health, pharmaceutics, agriculture, hydrology, geospatial mapping and animal husbandry experts. The success of this coordination indicates the level of associated political support, but as explained above, this was not always a given. Researchers have also highlighted the impact of farming practices, population movement and China's economic progress on control of this disease (44). In the 1950s, hundreds of millions in 12 southern provinces were at risk of this disease, and around 2% of China's population was infected (45,46). Early control efforts focused on transmission control, especially by early mass mobilization of people to alter snail habitats (45). With the introduction of praziquantel in the early 1980s (47) the focus changed to morbidity control, and mass treatment funded by a World Bank loan and other activities from 1992–2000 (45). In each case the observed reduction in infection numbers was at risk when priority and funding for control programs declined (46). After completion of the World Bank project, case numbers rose again in certain areas (48); the concentration of cases in poor rural areas and the lack of funding for preventive health care in general led to diminished control efforts, leading national health authorities to rate schistosomiasis control, tuberculosis, hepatitis B and HIV as equally critical priorities, in contrast to its status as a neglected tropical disease in other nations (49). Schistosomiasis persists in seven provinces, in a much smaller area of the upper and lower Yangtze River catchment and particularly in villages whose population totals around three million people (41). National funding was required to kick-start new control efforts including periodic mass chemotherapy, reduction of infection sources (animal management, mechanization of farming, water supply and sanitation measures) and public education, supported by a 2004–2015 government-funded vertical project (49–51). Based on infection rates among the population and cattle in the affected areas, this is apparently the most successful combination of activities yet, and the screening program being undertaken has also demonstrated an impact on rates of infection with the soil-transmitted helminths *Ascaris lumbricoides* and *Trichuris trichiuria*, probably as a result of the sanitation and public education components (51).

Tuberculosis – persistently high case numbers despite effective diagnosis and treatment

TB is probably the most important communicable disease that China has struggled to control. China has the world's second highest number of cases of TB (after India) and accounts for 16% of the world's disease burden. It is estimated that around 45% of the population are infected with *Mycobacterium tuberculosis*, with rates of infection and active disease much higher in rural and western areas; cases number around 1.5 million per year, and deaths around 160 000. Again, TB has been the focus of several large externally-funded projects in China over the last two decades, focusing especially on the introduction and expansion of the five-component Directly Observed Treatment (Short-Course) or DOTS strategy promoted by the World Health Organisation. These were effective in treating patients identified and appropriately referred to dedicated TB facilities, but relatively ineffective in improving case-detection and suffered from many of the same problems as the immunization and schistosomiasis programs. Several reports concluded that there were socio-economic barriers to care-seeking, failure or delay in referring patients for available free treatment (due to loss of income by referring clinicians), weak coordination between hospitals and public health authorities and weak local political and financial prioritization of TB case detection and management (that is, weak co-funding), particularly in poorer counties (52,53). The nature of TB as a disease affecting the poor, the itinerant and those least able to pay for treatment applies in China as elsewhere; absolute case numbers have increased with the population and the problem of multi-drug resistance, currently around 8% of cases, is rising.

Overall TB control in China was another example where CDC suffered due to lack of public funding in poor areas, marketisation of the health sector resulting in lack of patient access to free care, and its handling as a vertical rather than integrated clinical-and-public-health program. More recently, in the context of an overall improvement in CDC in China since 2003, massively increased national funding and improved surveillance for disease using the internet have enabled China to meet and maintain global

TB control targets of detecting at least 70% of new sputum-smear positive cases and curing 85% of them (32,54,55). As with schistosomiasis, the increase in national funding for TB control is very encouraging. However, the same challenges continue to apply to TB control as to CDC and public health in China in general: national and local funding for dedicated and trained staff and services, and making related services accessible and affordable to all, including the mobile population, despite the continued focus on profit in most health facilities.

Control of sexually-transmitted diseases – China's newest vertical CDC program

By contrast to the targets of vertical disease-control initiatives, sexually transmitted diseases (STDs) have re-emerged as a major priority in China due to the lack of such a program. China's legendary success in controlling STDs during the 1950s and 1960s was due to a combination of socialization (in which STDs were portrayed not typically as a sign of "bad behaviour" but as a legacy of the old bourgeois and exploitative society, particularly with respect to women); treatment (destigmatising syphilis and gonorrhoea made mass screening and drug treatment relatively easy), and socio-economic approaches (the banning of prostitution, emancipation of women and creation of employment for poor women) (4,6,10,11). This combination was inseparable from the revolutionary milieu of the time, and despite very high rates of infection during the early years of the People's Republic, helped to "eliminate" STDs from China by 1964 (10,56). This situation prevailed until the liberalization of commerce, movement, social customs and secular changes in sexual behaviour allowed the reappearance of STDs in the 1980s (39,56). There were massive increases in STD incidence and an emerging HIV problem in China in the 1990s (57), and the same problems that have led to difficulties in sustaining control of TB and schistosomiasis have plagued STD control: lack of knowledge of disease prevention and treatment, including HIV, among the poor and some echelons of the health sector (58); lack of physical and financial access to good care, along with profiteering by health providers; lack of funding for screening programs, and poor coordination across sectors (including within the health sector, between MCH staff and other clinicians), creating an urgent problem (59,60). According to a former director at China's National Centre for Women's and Children's Health, in 2008: "In the past fifteen years, the prevalence of congenital syphilis increased by 2000 times in China, excluding foetal deaths, stillbirths and abortions caused by syphilis during pregnancy. Surveillance data reveal the incidence of congenital syphilis increased at the rate of 72% each year from 0.01 in 1991 to 35 in 2006 per 100000 live births" (Wang Linhong, former Director, National Centre for Women's and Children's Health at China CDC, personal communication). Syphilis is now numerically the third most common reportable infectious disease in the PRC, behind viral hepatitis and TB (Dr Yang Weizhong, China CDC, personal communication). To its credit, the government has again massively increased funding for education, screening and treatment of STDs, including HIV (60), but the long term success of these measures will again depend on the level of uptake of these activities, fair access to care and local government support.

CHINA'S WAKE-UP CALL ON CDC: IMPROVEMENTS SINCE SARS AND HEALTH SYSTEM REFORM

For both TB and schistosomiasis, it is evident that cessation of internally- and externally-supported disease control programs in the early 2000s was a major setback. Outside the academic and public health community in China, interest to fund and implement programs to control specific diseases associated with poverty and under-development was low at this time. As a result, despite improvements in nutrition, socio-economic status and health infrastructure, there was little progress in infectious disease rates and suggestions that some were increasing slightly during this period (30), although it is likely that this also reflected improved surveillance and diagnosis (39). What was undoubted, however, was the increasing urgency of major reform to CDC and China's health sector in general (29,61–63) due to worsening equity (21, 64–66), a high level of public complaint and government acknowledgement of the problem. Crystallising the situation in the most humbling way came the SARS outbreak in early 2003, which forced China's government and health authorities to act quickly and decisively on the dangerous situation with respect to CDC and, albeit more slowly, on the reform of the health sector.

Much has been written about China's initial denial of the extent of the SARS outbreak (67), and the implications for its control (68). The events occurred despite preceding attempts to renovate the EPSs, as described above, but there is no denying that China remained grossly ill-equipped to deal with a disease of this nature in 2003, and government hugely increased its support for CDC (physical infrastructure, staffing and funding) after this shock (39). Two other major CDC-related impacts of SARS in China were undertaken. First was the revision of the Law on Infectious Diseases in August 2004, mandating the reporting of 37 notifiable conditions, including immediate reporting of certain diagnoses and replacing a system which had essentially become optional and mainly answerable to local government, not the CDC hierarchy. As a result, in restoring its population health objectives CDC was mainstreamed in China's health sector, with both the curative and disease-control

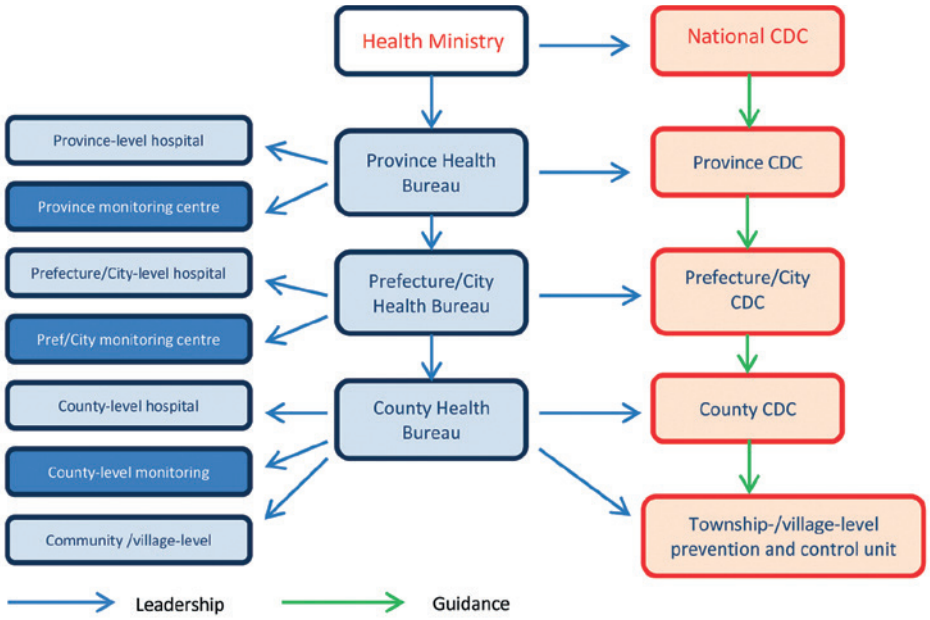


Figure 4 China's new infectious disease prevention and control system, introduced in 2004 (after Yang Weizhong, personal communication, 2010).

sectors responsible for prevention, reporting and management of infectious diseases (Dr Yang Weizhong, China CDC, personal communication) (**Figure 4**).

Second was the development of a new electronic notifiable disease reporting system to answer the central government's request for a case-based, national, integrated and web-based system (incorporating notifiable diseases, risk factors, emergencies and also specific systems for reporting certain diseases like TB, influenza, plague and HIV). In contrast to the old system of weekly and monthly consolidated reports, the new system uses the internet rather than email to upload disease information, not only from local CDC facilities but also from hospitals and health inspection agencies, enabling analysis of data pertaining to reportable diseases and identification of disease outbreaks and trends in real time (**Figure 5**).

Again, the mandating of hospital reporting drew the clinical sector into CDC as never before, raising clinicians' awareness on the public health significance of their actions on infectious diseases and population health; the coverage of this reporting system in 2009 was 100% of CDC-facilities, 97.8% of county-level or higher hospitals and 83.8% of township/village-level facilities, up from 66% in 2007, and the delay in reporting of and entering a notifiable disease report is reported to have dropped from almost 5 and 3.5 days, respectively, to less than one day (Dr Yang Weizhong, China CDC, personal communication). Additional surveillance continues through the notifiable disease reporting system and specific surveillance systems for HIV/AIDS and other STDs, TB, EPI target diseases (for example, for acute flaccid paralysis and measles) and others. The impact of these two initiatives is evident in the rise in the number of notifiable disease reports since 2003 (39) (**Figure 6**).

Alongside these two broad CDC initiatives, a number of disease-specific, donor- and particularly government-funded initiatives have also demonstrated an increased commitment to CDC in China. These include massive increases in funding for control of TB, schistosomiasis, malaria and STDs; treatment and prevention of maternal-to-child-transmission of HIV/AIDS; prevention, screening and treatment of other STDs; vaccine-preventable diseases (such as control of measles through various provincial campaigns and a national campaign in September 2010; control of hepatitis B through catch-up vaccination of older children; expansion of routine immunization to cover 12 antigens since 2007; an enormous program of subsidies to encourage hospital delivery and prevention of neonatal tetanus (also enabling dramatic increases in birth-dosing with hepatitis B vaccine) and introduction of a national child immunisation registration and information system); infectious disease surveillance during emergencies (including use of mobile phones to report on disease incidence in the areas affected by the Sichuan earthquake) and public education campaigns and research to reduce the risk of emerging threats such as recrudescence of dengue fever; increases in brucellosis, zoonoses and the impact of annual outbreaks of influenza and EV71 infection (data available upon request). Both GAVI and the Global Fund for AIDS, TB and Malaria have also supported large scale CDC activities in China in recent years.

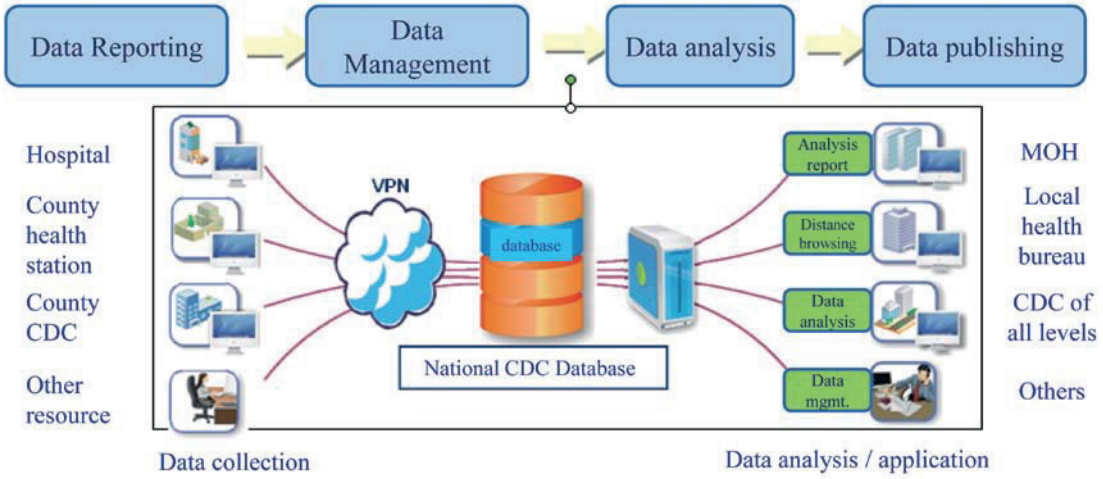


Figure 5 China's web-based notifiable infectious disease direct reporting system (Yang Weizhong, China CDC, permission to reproduce received).

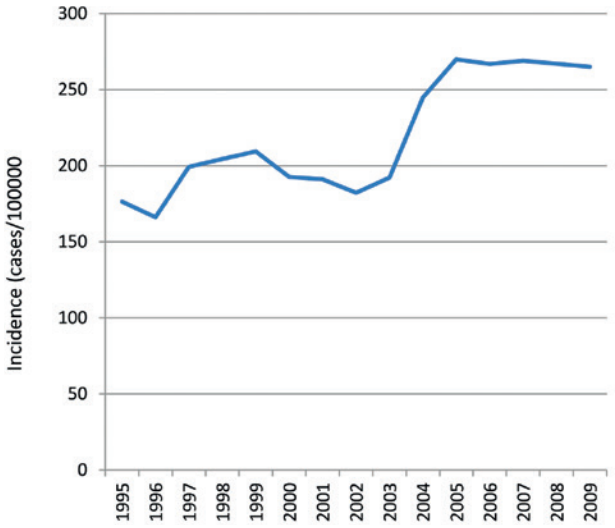


Figure 6 Improved notifiable disease reporting since introduction of real-time system in 2004 (after Wang (39) and data presented at an International Symposium on Research and Control of Infectious Diseases of Poverty, Shanghai, China, 2010).

These developments have since 2009 been taking place in the context of other major developments in China's health sector, some of which are likely to directly benefit CDC. Among the initiatives being rolled out as part of China's health system reform (HSR) are a 15 (now 25) yuan-per-capita public health subsidy for grassroots-level providers, to facilitate their implementation of nine public health activities at village level; including health promotion and implementation of CDC; a National Essential Drugs Scheme intended to control prescribing practices and profiteering by village and township doctors, including in the treatment of infectious diseases (26), and even more funding to improve the staffing and physical infrastructure of China's health system (69).

RISKS AND CHALLENGES

There is no doubt that China is in a much better position to handle another disease outbreak like SARS; indeed, the response to the ongoing highly-pathogenic H5N1 and 2009 H1N1 influenza outbreaks, despite accusations of under-reporting and heavy-handed quarantine of travellers, demonstrate China's increased capacity and intention to act quickly, decisively and in unison across national, provincial and county levels on CDC when population health is threatened.

In fact, the major reasons for slow progress in some aspects of CDC overall is not unique to CDC, nor to China. Decentralisation of the funding and implementation of many health programs in China and elsewhere, although forced upon governments by economic reality and the need to build capacity and encourage the taking of responsibility, is inimical to consistent, reliable and robust outcomes. To the extent that China is relying on poor, predominantly rural provinces and counties for CDC, the wait for elimination of infectious diseases dependent on more than drugs and vaccines may be a long one.

Another problem, also not unique to China but perhaps less tolerable in a nation of its size and importance to global health, is the opacity of the situation at certain times. Despite marked improvements in disease surveillance and CDC since SARS, a remarkably similar and concerning reluctance to report disease outbreaks in times of political sensitivity persists. Recent examples include the likely cover-up of the

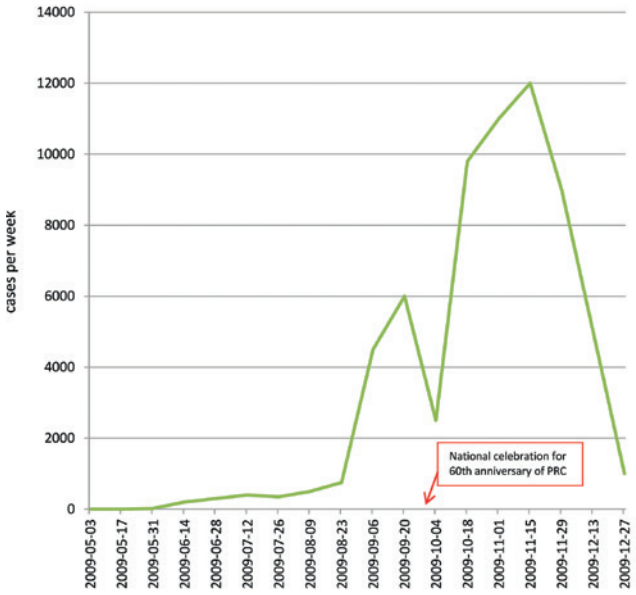


Figure 7 H1N1 case numbers in China by fortnight, May – December 2009 (based on data presented at an International Symposium on Research and Control of Infectious Diseases of Poverty, Shanghai, China, 2010). PRC – People's Republic of China.

melamine scandal before the Olympic Games in 2008 (70), and the probable under-reporting of cases of H1N1 influenza just prior to the celebration of the 60th anniversary of the People's Republic in October 2009 (**Figure 7**). The ongoing tendency of those in power in China to put nationalism and politics ahead of public health at certain key times suggests a continuing risk for CDC (71).

China has not yet taken up global recommendations to vaccinate all children against rotavirus, *Pneumococci, Haemophilus influenza* type b and human papillomavirus. Although the national incidence of rotavirus diarrhoea is almost certainly lower than previously thought (72), a case could easily be made for introduction of the vaccine in poorer provinces or in rural areas, on mortality, morbidity and possibly economic grounds. The same could be said for the two respiratory pathogens, but the data is scant and there has been a long-standing reluctance to introduce these vaccines in China, for two reasons: first, given that China does not use any of the newer combination vaccines it will further complicate an already-crowded vaccination schedule; second and more important, local manufacture of most of these vaccines has not yet commenced, and China does not use imported vaccines in its EPI. These and other so-called category B vaccines are available for private purchase from CDC facilities across China, but there are no data on coverage. It is safe to assume that those who would benefit from them most do not receive them.

Other risks for CDC in China have recently been studied by experts and some are perceived to remain significant. These include the risk of population mobility, persistent proximity of humans and animals in some areas, the regular appearance of new strains of influenza and other pathogens in China, behaviour changes impacting on STDs and the continued low standard of clinical care in poor areas (68,73).

Finally, TB is not the only bacterium for which antibiotic resistance is a major emerging problem in China. Marketisation of the health sector, all the way down to village level, resulted in massive overuse of antibiotics, and a very active pharmaceutical manufacturing sector has avidly promoted "new, improved" drugs to health providers across the nation. Although data are hard to come by as clinical microbiology is a luxury not usually purchased by health services in China, it is safe to assume that multi-resistant bacteria are common in China, and pose a threat to CDC in clinical settings.

CONCLUSION

The study of CDC in China provides a fascinating opportunity to understand the early tribulations and achievements of the People's Republic, during which time the top-down campaign-style approaches adopted from the Soviet Union were replaced by a bottom-up approach led by village doctors, supported by township and county cadres and funded by the CMS. The introduction of a market economy, with the breakdown of these grassroots structures and the reliance on vertical programs has challenged CDC in China. Changes to reporting and the structure and priority of CDC after SARS, along with more recent reforms of the health sector and injection of new funds for disease control programs, allows reasonable expectations of further progress in CDC in the world's largest nation.

Acknowledgements: Dr Yang Weizhong, Deputy Director, China CDC, provided permission for reproduction of a slide used in a presentation given at an International Symposium on Research and Control of Infectious Diseases of Poverty in Shanghai, China, June, 2010, as Figure 5. Various facts from this presentation are also cited as personal communications in this paper. The author is grateful for the support of Drs Guo Sufang and Zhu Xu at UNICEF China in preparation of this paper, and to Drs Grant Miller and others at the University of California, Stanford, for the permission to cite their unpublished work as ref. 8, and Dr Yang Li and others at China CDC and elsewhere for the permission to cite their unpublished work as ref. 26.

Funding: This article was prepared based on work undertaken whilst the author was employed by UNICEF China, with additional support from the Australian Society for Infectious Disease and the University of Edinburgh.

Ethical approval: Not required.

Competing interests: The author has completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declares: No support from any organization for the submitted work; no financial relationships were declared with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

1. Banister J. China's changing population. Stanford, USA: Stanford University Press; 1987.
2. Hesketh T, Lu L, Xing ZW. The effect of China's one-child family policy after 25 years. N Engl J Med. 2005;353:1171–1176.
3. Jamison DT, Evans JR, King T, Porter I, Prescott N, Prost A. China: The health sector. Washington DC: World Bank;1984.
4. Cook IG, Dummer TJ. Changing health in China: re-evaluating the epidemiological transition model. Health Policy. 2004;67:329–343.
5. Sidel VW. The barefoot doctors of the People's Republic of China. N Engl J Med. 1972;286:1292–1300.
6. Bien CH. The Barefoot Doctors: China's rural health care revolution,1968–1981 [Honors Thesis]. [Middletown, CT]: Wesleyan University; 2008.
7. Xu X. Control of communicable diseases in the People's Republic of China. Asia Pac J Public Health. 1994;7:123–131.
8. Miller G, Eggleston KN, Zhang Q. Understanding China's mortality decline under Mao: A provincial analysis, 1950–1980. Unpublished manuscript; 2010.
9. Abrams HK. The resurgence of sexually transmitted disease in China. J Public Health Policy. 2001;22:429–440.
10. Cohen MS, Henderson GE, Aiello P, Zheng H. Successful eradication of sexually transmitted diseases in the People's Republic of China: implications for the 21st century. J Infect Dis. 1996;174 Suppl 2:S223–229.
11. Tucker JD, Chen X–S, Peeling RW. Syphilis and social upheaval in China. N Engl J Med. 2010;362:1658–1661.
12. Lee PR. Medicine and public health in the People's Republic of China. West J Med. 1974;120:430–437.
13. Weiyuan C. China's village doctors take great strides. Bull World Health Organ. 2008;86:914–915.
14. Hsiao WC. Transformation of health care in China. N Engl J Med. 1984;310:932–936.
15. Liu XZ, Wang JL. An introduction to China's health care system. J Public Health Policy. 1991;12:104–116.
16. The Government of the Peoples' Republic of China. Guiding opinions of the General Office of the State Council on further strengthening the building of a workforce of village doctors. Beijing: Government of China; 2011.
17. Diep KN. Diagnosing the revolution: Medical reform and the barefoot doctor program in the Chinese Cultural Revolution, 1966–1976 [Honors Thesis]. [Cambridge, MA]: Harvard University; 2010.
18. Hillier S, Zheng, X. Rural health care in China: past, present and future. In: Dwyer DJ, ed. China: the next decades. Harlow: Longman Scientific & Technical; 1994. p. viii, 263 p.
19. Liu Y, Hsiao WC, Li Q, Liu X, Ren M. Transformation of China's rural health care financing. Soc Sci Med. 1995;41:1085–1093.
20. Bloom G, Xingyuan G. Health sector reform: lessons from China. Soc Sci Med. 1997;45:351–360.
21. Blumenthal D, Hsiao W. Privatization and its discontents--the evolving Chinese health care system. N Engl J Med. 2005;353:1165–1170.
22. Zhan SK, Tang SL, Guo YD, Bloom G. Drug prescribing in rural health facilities in China: implications for service quality and cost. Trop Doct. 1998;28:42–48.
23. Pavin M, Nurgozhin T, Hafner G, Yusufy F, Laing R. Prescribing practices of rural primary health care physicians in Uzbekistan. Trop Med Int Health. 2003;8:182–190.

24. Dong L, Yan H, Wang D. Antibiotic prescribing patterns in village health clinics across10 provinces of Western China. J Antimicrob Chemother. 2008;62:410–415.
25. Chen W, Tang S, Sun J, Ross-Degnan D, Wagner AK. Availability and use of essential medicines in China: manufacturing, supply, and prescribing in Shandong and Gansu provinces. BMC Health Serv Res. 2010;10:211.
26. Li YCY, Brant P, Sufang G, Bin L, Hipgrave D. Introduction of the new National Essential Medicines System in China: Policy implementation and financial impact. Unpublished manuscript.
27. Brixi H, Mu Y, Targa B, Hipgrave D. Equity and public governance in health system reform: Challenges and opportunities for China. Policy Research Working Paper N0. 5530. Washington, DC: The World Bank; 2010.
28. China Institute for Reform and Development and United Nations Development Program. China human development report, 2007. Beijing: China; 2007.
29. The World Bank. China's health sector – why reform is needed. Rural health in China: Briefing notes series. Washington DC: The World Bank; 2005.
30. Dummer TJ, Cook IG. Exploring China's rural health crisis: processes and policy implications. Health Policy. 2007;83:1–16.
31. The World Health Organisation. Primary health care – Now more than ever. Geneva, Switzerland: WHO; 2008.
32. Wang L, Liu J, Chin DP. Progress in tuberculosis control and the evolving public-health system in China. Lancet. 2007;369:691–696.
33. Liu Y. China's public health-care system: facing the challenges. Bull World Health Organ. 2004;82:532–538.
34. Liu X, Mills A. Financing reforms of public health services in China: lessons for other nations. Soc Sci Med. 2002;54:1691–1698.
35. Zeng G, Zhang JK, Rou KM, Xu C, Cheng YK, Qi GM. Infectious disease surveillance in China. Biomed Environ Sci. 1998;11:31–37.
36. Chen C. Disease surveillance in China. Morbidity and Mortality Weekly Report. 1992;111 (Dec 1992).
37. Peng J, Zhang SN, Lu W, Chen AT. Public health in China: the Shanghai CDC perspective. Am J Public Health. 2003;93:1991–1993.
38. Walgate R. China sets up Centres for Disease Control and Prevention. Bull World Health Organ. 2002;80:335.
39. Wang L, Wang Y, Jin S, Wu Z, Chin DP, Koplan JP, et al. Emergence and control of infectious diseases in China. Lancet. 2008;372:1598–1605.
40. Ministry of Foreign Affairs of the People's Republic of China, United Nations System in China. China's progress towards the Millennium Development Goals. Beijing, China: Ministry of Foreign Affairs; 2008.
41. Montresor A, Cong DT, Sinuon M, Tsuyuoka R, Chanthavisouk C, Strandgaard H, et al. Large-scale preventive chemotherapy for the control of helminth infection in Western Pacific countries: six years later. PLoS Negl Trop Dis. 2008;2:e278.
42. Cao Q, Jarrett SW, Parker RL, Taylor CE, Yang BP. Immunizing the children of China. Assignment Children. 1988;69:347–360.
43. Ministry of Health of the People's Republic of China. The Report of National EPI Review in 2004. Beijing, China: Ministry of Health; 2005.
44. Huang YX, Manderson L. The social and economic context and determinants of schistosomiasis japonica. Acta Trop. 2005;96:223–231.
45. Utzinger J, Zhou XN, Chen MG, Bergquist R. Conquering schistosomiasis in China: the long march. Acta Trop. 2005;96:69–96.
46. Zhou XN, Wang LY, Chen MG, Wu XH, Jiang QW, Chen XY, et al. The public health significance and control of schistosomiasis in China--then and now. Acta Trop. 2005;96:97–105.
47. Xiao SH, Keiser J, Chen MG, Tanner M, Utzinger J. Research and development of antischistosomal drugs in the People's Republic of China a 60-year review. Adv Parasitol. 2010;73:231–295.
48. Liang S, Yang C, Zhong B, Qiu D. Re-emerging schistosomiasis in hilly and mountainous areas of Sichuan, China. Bull World Health Organ. 2006;84:139–144.
49. Wang L, Utzinger J, Zhou XN. Schistosomiasis control: experiences and lessons from China. Lancet. 2008;372:17931795.
50. Wang LD, Chen HG, Guo JG, Zeng XJ, Hong XL, Xiong JJ, et al. A strategy to control transmission of Schistosoma japonicum in China. N Engl J Med. 2009;360:121–128.
51. Wang LD, Guo JG, Wu XH, Chen HG, Wang TP, Zhu SP, et al. China's new strategy to block Schistosoma japonicum transmission: experiences and impact beyond schistosomiasis. Trop Med Int Health. 2009;14:1475–1483.
52. Zhan S, Wang L, Yin A, Blas E. Revenue-driven in TB control – three cases in China. Int J Health Plann Manage. 2004;19 Suppl 1:S63–78.
53. Tang S, Squire SB. What lessons can be drawn from tuberculosis (TB) control in China in the 1990s? An analysis from a health system perspective. Health Policy. 2005;72:93–104.
54. The World Health Organisation. Global tuberculosis control: a short update to the 2009 report. Geneva: WHO; 2009.
55. Wang L, Liu X, Huang F, Hennig C, Uplekar M, Jiang S. Engaging hospitals to meet tuberculosis control targets in China: using the Internet as a tool to put policy into practice. Bull World Health Organ. 2010;88:937–942.
56. Parish WL, Laumann, E.O., Mojola, S. Sexual behavior in China: Trends and comparisons. Population and Development Review. 2007;33:729–756.

REFERENCES

57. Chen XS, Gong XD, Liang GJ, Zhang GC. Epidemiologic trends of sexually transmitted diseases in China. Sex Transm Dis. 2000;27:138–142.
58. Guan J, Wu Z, Li L, Lin C, Rotheram-Borus MJ, Detels R, et al. Self-reported sexually transmitted disease symptoms and treatment-seeking behaviors in China. AIDS Patient Care STDS. 2009;23:443448.
59. Tucker JD, Hawkes SJ, Yin YP, Peeling RW, Cohen MS, Chen XS. Scaling up syphilis testing in China: implementation beyond the clinic. Bull World Health Organ. 2010;88:452–457.
60. Tucker JD, Cohen MS. China's syphilis epidemic: epidemiology, proximate determinants of spread, and control responses. Curr Opin Infect Dis. 2011;24:50–55.
61. Guo Y, Shibuya K, Cheng G, Rao K, Lee L, Tang S. Tracking China's health reform. Lancet. 2010;375:1056–1058.
62. Wang H, Ge, Y., Gong, S. Regulating medical services in China. New York: Milbank Memorial Fund and Department of Social Development Development Research Center (DRC) The State Council of P.R. China; 2007.
63. The World Health Organisation, China and Social Development Department of China State Council Development Research Centre. China: Health, poverty and economic development. Beijing, China; 2006.
64. World Health Organisation. The world health report 2000: Health systems: improving performance. Geneva: WHO; 2000.
65. Wang H, Xu T, Xu J. Factors contributing to high costs and inequality in China's health care system. JAMA. 2007;298:1928–1930.
66. United Nations Health Partners Group in China. A health situation assessment of the People's Republic of China. Beijing: United Nations;2005.
67. Fleck F. How SARS changed the world in less than six months. Bull World Health Organ. 2003;81:625–626.
68. Kaufman J. Infectious disease challenges in China. In: Freeman CW, Lu, X.Q., editor. China's capacity to manage infectious diseases. Washington, DC: Center for Strategic and International Studies; 2009.
69. Chen Z. Launch of the health-care reform plan in China. Lancet. 2009;373:1322–1324.
70. Yardley J, Barboza, D. Lapses let tainted milk flow. New York Times. 2008 26 September, 2008.
71. Anonymous. China: towards "xiaokang", but still living dangerously. Lancet. 2004;363:409.
72. Yee EL, Fang ZY, Liu N, Hadler SC, Liang X, Wang H, et al. Importance and challenges of accurately counting rotavirus deaths in China, 2002. Vaccine. 2009;27 Suppl 5:F46–49.
73. Nicoll A, Huang J, Xie Z. Foresight Infectious Diseases China Project – a novel approach to anticipating future trends in risk of infectious diseases in China: methodology and results from an initial application. Euro Surveill. 2009;14. pii: 19261.

Il terzo coronavirus del nuovo secolo: SARS-COV-2

Fare il punto della situazione in tempo reale su un'epidemia in corso nelle fasi iniziali è molto difficile, ma l'attenzione del mondo scientifico nei confronti di SARS-COV-2, il terzo nuovo coronavirus del millennio, ha imposto una grande accelerazione nella sintesi delle evidenze disponibili, con constanti aggiornamenti.

I primi report hanno evidenziato le tappe cruciali che hanno caratterizzato l'epifania della nuova epidemia: l'identificazione di un primo cluster di polmoniti atipiche a fine dicembre 2019 nella città di Wuhan; l'annuncio dell'isolamento di un nuovo coronavirus da parte delle autorità cinesi l'8 gennaio 2020; la condivisione della prima sequenza del genoma virale il 10 gennaio, effettuata da ricercatori di Shanghai; il primo caso identificato al di fuori dei confini cinesi, il 13 gennaio in Tailandia.

Riferimento bibliografico

Gralinski LE et al. Return of the Coronavirus: 2019-nCoV. Viruses 2020; 12: 135.

Segue articolo full text originale

viruses

MDPI

Commentary

Return of the Coronavirus: 2019-nCoV

Lisa E. Gralinski [1] and Vineet D. Menachery [2,3,*]

1 Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC 27514, USA; lgralins@email.unc.edu
2 Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555, USA
3 Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX 77555, USA
* Correspondence: Vimenach@utmb.edu

Received: 21 January 2020; Accepted: 24 January 2020; Published: 24 January 2020

Abstract: The emergence of a novel coronavirus (2019-nCoV) has awakened the echoes of SARS-CoV from nearly two decades ago. Yet, with technological advances and important lessons gained from previous outbreaks, perhaps the world is better equipped to deal with the most recent emergent group 2B coronavirus.

Keywords: 2019-nCoV; novel CoV; Wuhan; Wuhan pneumonia; coronavirus; emerging viruses; SARS-CoV; MERS-CoV

1. Emergence

The third zoonotic human coronavirus (CoV) of the century emerged in December 2019, with a cluster of patients with connections to Huanan South China Seafood Market in Wuhan, Hubei Province, China. Similar to severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) infections, patients exhibited symptoms of viral pneumonia including fever, difficulty breathing, and bilateral lung infiltration in the most severe cases [1]. News reports of patients with an unknown pneumonia were first identified on 31st December with the Wuhan Municipal Health Commission saying they were monitoring the situation closely (Figure 1). On 1st January 2020, the seafood market was closed and decontaminated while countries with travel links to Wuhan went on high alert for potential travelers with unexplained respiratory disease. After extensive speculation about a causative agent, the Chinese Center for Disease Control and Prevention (CDC) confirmed a report by the Wall Street Journal and announced identification of a novel CoV on 9th January [2]. The novel CoV (2019-nCoV) was isolated from a single patient and subsequently verified in 16 additional patients [3]. While not yet confirmed to induce the viral pneumonia, 2019-nCoV was quickly predicted as the likely causative agent.

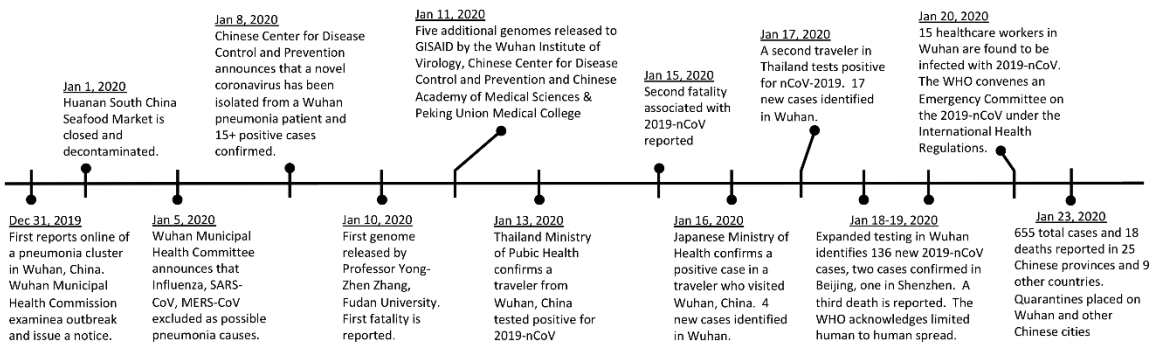


Figure 1. Timeline of the key 2019-nCoV events.

The first sequence of 2019-nCoV was posted online one day after its confirmation on behalf of Dr. Yong-Zhen Zhang and scientists at Fudan University, Shanghai [4]. Subsequently, five additional 2019-nCoV sequences were deposited on the GSAID database on 11th January from institutes across China (Chinese CDC, Wuhan Institute of Virology and Chinese Academy of Medical Sciences & Peking Union Medical College) and allowed researchers around the world to begin analyzing the new CoV [5]. By 17th January, there were 62 confirmed cases in China and importantly, three exported cases of infected travelers who were diagnosed in Thailand (2) and Japan (1) [6]. The sequences of these exported cases and several additional 2019-nCoV isolated in China have also been deposited on the GSAID database [5]. Diagnostic tests have subsequently been developed and some are being used on suspect cases identified in other locations including Vietnam, Singapore, and Hong Kong [7]. To date there have been twenty-six fatalities associated with 2019-nCoV infection, many of these cases had significant co-morbidities and were older in age (>50). A range of disease has been observed highlighted by fever, dry cough, shortness of breath, and leukopenia; patients have included mild cases needing supportive care to severe cases requiring extracorporeal membrane oxygenation; however, compared to SARS-CoV (10% mortality) and MERS-CoV (35% mortality), the 2019-nCoV appears to be less virulent at this point with the exception of the elderly and those with underlying health conditions. Initial monitoring of case close contacts had not revealed any further 2019-nCoV cases. However, modeling analysis based on official case numbers and international spread suggested that there may be cases going undetected [8]. On 19th January, these fears were seemingly confirmed as an additional 136 cases were added from further surveys raising the total in Wuhan to 198 infected patients [9]. Among the 198 total cases in Wuhan, 170 remained in hospitals, 126 mostly with mild symptoms, 35 in serious condition, and 9 in critical condition. The expanded numbers and extended range of onset dates (12 December 2019–18 January 2020) suggested likely human to human transmission or ongoing transmission from a market or other primary sources. On 20th January, the outbreak was further expanded to other parts of China (Beijing, Shanghai, & Shenzhen) as well as another exported cases to South Korea. As of January 24, the total case number has expanded to at least 870 total cases with 26 deaths across 25 provinces in China and 19 exported cases in 10 countries [10]. Public health authorities have quarantined travel from Wuhan to limit the spread of the virus and reports indicate other Chinese cities have also been isolated [11]. With the heavy travel season for lunar New Year underway in Asia, major concerns exist for the 2019-nCoV outbreak to continue and spread.

2. Origins of 2019-nCoV

The source of the 2019-nCoV is still unknown, although the initial cases have been associated with the Huanan South China Seafood Market. While many of the early patients worked in or visited the market, none of the exported cases had contact with the market, suggesting either human to human transmission or a more widespread animal source [6]. In addition to seafood, it is reported on social media that snakes, birds and other small mammals including marmots and bats were sold at the Huanan South China Seafood Market. The WHO reported that environmental samples taken from the marketplace have come back positive for the novel coronavirus, but no specific animal association has been identified [6]. An initial report suggested that snakes might be the possible source based on codon usage [12], but the assertion has been disputed by others [13]. Researchers are currently working to identify the source of 2019-nCoV including possible intermediate animal vectors.

A zoonotic reservoir harkens back to the emergence of both SARS- and MERS-CoV. SARS-CoV, the first highly pathogenic human CoV, emerged in 2002 with transmission from animals to humans occurring in wet markets. Surveillance efforts found SARS-CoV viral RNA in both palm civets and raccoon dogs sold in these wet markets [14]; however, SARS-CoV was not found in the wild, suggesting that those species served as intermediary reservoir as the virus adapted to more efficiently infect humans. Further surveillance efforts identified highly related CoVs in bat species [15]. More recent work has demonstrated that several bat CoVs are capable of infecting human cells without a need for intermediate adaptation [16,17]. Additionally, human serology data shows recognition of bat CoV

proteins and indicates that low-level zoonotic transmission of SARS-like bat coronaviruses occurs outside of recognized outbreaks [18]. MERS-CoV is also a zoonotic virus with possible origins in bats [19,20], although camels are endemically infected and camel contact is frequently reported during primary MERS-CoV cases [21]. For SARS-CoV, strict quarantine and the culling of live markets in SE Asia played a major role in ending the outbreak. With the cultural importance of camels, a similar approach for MERS-CoV was not an option and periodic outbreaks continue in the Middle East. These lessons from SARS and MERS highlight the importance of rapidly finding the source for 2019-nCoV in order to stem the ongoing outbreak.

3. Susceptible Populations

With limited patient data, it is difficult to make robust declarations about populations that may be most susceptible to 2019-nCoV. However, disease severity following SARS- and MERS-CoV corresponded strongly to underlying host conditions including age, biological sex, and overall health [22]. Early patient reports from 2019-nCoV find similar trends. Severe illness with 2019-nCoV has been associated with elderly patients (>60 years old), including twenty-six lethal cases. These findings correspond to increased severity and death in people over the age of 50 following both SARS and MERS-CoV infection [23,24]. Similarly, the underlying health of the patient likely plays a critical role in overall susceptibility. For the 2019-nCoV, limited comorbidity data is available; however, the twenty-six patients that have succumbed to the novel CoV had significant health conditions including hypertension, diabetes, heart and/or kidney function issues that may have made them more susceptible. For the MERS-CoV outbreak, smoking, hypertension, diabetes, cardiovascular disease, and/or other chronic illnesses have been present in the majority of deaths and correspond to findings in animal models [25]. The results indicate vigilance is necessary for these vulnerable patients following 2019-nCoV infection.

4. Insights from the 2019-nCoV Sequence

The rapid sequencing of the nearly 30,000 nucleotide 2019-nCoV genome by Dr. Zhang's group at Fudan University and several other groups in China illustrate the dedication and increased capacity of the scientific infrastructure in China [4,5]. For SARS-CoV, the causative agent was unknown for months and subsequently took over four weeks until a full genome was released [26]. Similarly, MERS-CoV was only identified after several months of testing and a full-length genome available about a month later [27]. In contrast, time from the first date of patient onset (12 December 2019) to the report of several 2019-nCoV full-length genomes took less than one month. Combined with the immense pressure of an ongoing outbreak with an unknown agent, the effort of these scientists should be considered nothing less than remarkable.

Building from the sequence, the nucleotide alignment quickly distinguished the novel virus as a group 2B CoV, distinct from the SARS-CoV strains [4,5]. Examining the whole genome, 2019-nCoV maintains ~80% nucleotide identity to the original SARS epidemic viruses. Its closest whole genome relatives are two bat SARS-like CoVs (ZC45 and ZXC21) that shared ~89% sequence identity with 2019-nCoV; these CoV sequences were deposited in early 2018 from Zhejiang province in *R. sinicus* bats in China. Comparing across the deposited 2019-nCoV strains finds > 99.5% conservation; the lack of diversity suggests a common lineage and source with emergence not likely having occurred that long ago [28,29]. A recent report has subsequently identified a bat CoV sequence, RaTG3, with 92% sequence identity with the novel virus which argues for bat origins for the 2019-nCoV [30].

We next shifted analysis to the nucleocapsid (N) protein, the most abundant protein produced in CoVs. Generally, the N protein is well conserved across CoV families including group 2B [31]. The N protein for 2019-nCoV is no exception with ~90% amino acid identity to the SARS-CoV N protein. While less conserved than other group 2B CoVs like HKU3-CoV and SHC014-CoV, 2019-nCoV antibodies against the N protein would likely recognize and bind the SARS-CoV N protein as well. N antibodies do not provide immunity to 2019-nCoV infection, but the cross reactivity with SARS-CoV N protein

would allow a serum based assay to determine exposure to the novel CoV in asymptomatic cases. While previous studies have found serum reactivity to group 2B virus N proteins in Chinese populations [18], exposure to 2019-nCoV should increase the dilution factor substantially if exposure/infection had occurred. Importantly, this information may provide insights about susceptibly and potential routes of spread through asymptomatic carriers.

Examining further, we next compared the spike proteins, the critical glycoprotein responsible for virus binding and entry. Overall, the 2019-nCoV spike protein has roughly 75% amino acid identity with SARS-CoV, which is less conserved than other group 2B CoVs including HKU3-CoV [31]. However, narrowing analysis to the spike receptor binding domain (RBD) of SARS-CoV (amino acids 318–518), the 2019-nCoV RBD is 73% conserved relative to the epidemic RBD. This conservation level places the 2019-nCoV RBD between HKU3-4 (62.7% conservation), a bat virus that cannot use human ACE2, and rSHC014 (80.8%), the most divergent bat CoV spike known to use human ACE2 for entry [16,32]. Importantly, the key binding residues for SARS-CoV have been identified [33]; among these fourteen residues predicted to interact directly with human ACE2, the receptor for SARS-CoV, eight amino acids are conserved in 2019-nCoV. Notably, several of these residues are also conserved relative to WIV1- and WIV16-CoV, two bat strains closely related to SARS-CoV and known to use human ACE2 [17,34]. Initial structural modeling suggest that the 2019-nCoV may be able to use human ACE2 as a receptor, although its affinity m be reduced relative to the epidemic SARS-CoV strains [35]. A subsequent report demonstrated that the receptor binding domain of 2019-nCoV was capable of binding ACE2 in the context of the SARS-CoV spike protein [36]. In addition, another rapid report links demonstrates 2019-nCoV uses ACE2 receptors from human, bat, civets, and swine [30]. Together, the modeling, pseudotyping, and infection data provide strong evidence for human ACE2 being the receptor for 2019-nCoV.

5. Achieving Koch Postulates

Traditional identification of a microbe as the causative agent of disease requires fulfillment of Koch's postulates, modified by Rivers for viral diseases [37]. At the present time, the 2019-nCoV has been isolated from patients, detected by specific assays in patients, and cultured in host cells (one available sequence is identified as a passage isolate), starting to fulfill these criteria. Given the recentness of the 2019-nCoV outbreak, at this point there is no animal model available to fulfill the remaining criteria: 1) testing the capability of 2019-nCoV to cause respiratory disease in a related species, 2) re-isolating the virus from the experimentally infected animal and 3) detection of a specific immune response. These efforts will surely be an area of intense research in the coming months both in China and in CoV research laboratories around the world.

Notably, generating small animal models of coronavirus disease can be difficult. While SARS-CoV readily infected laboratory mice, it does not cause significant disease unless the virus is passaged to adapt to the mouse host [38]. Infection of primates produces a more mild disease than that observed in humans, although fever and pulmonary inflammation were noted [39,40]. MERS-CoV is incapable of infecting rodent cells without engineering changes in critical residues of the receptor protein, DPP4 [41,42]. However, MERS-CoV does infect non-human primates [43]. As such, MERS mouse models of disease required a great deal of time to develop and are limited in the types of manipulations that can be performed [41]. At this point, the infectious capability of the 2019-nCoV for different species and different cell types is unknown. Early reports suggest that the virus can utilize human, bat, swine, and civet ACE2 [30]; notably, the group found mouse Ace2 was not permissive for 2019-nCoV infection Dissemination of virus stocks and/or de novo generation of the virus through reverse genetics systems will enable this research allowing for animal testing and subsequent completion of Koch's postulates for the new virus.

6. Threat for Spread: Human to Human, Health Care Workers, and Super Spreaders

While the Huanan seafood market in Wuhan has been associated with the majority of cases, many of the recent cases do not have a direct connection [9]. This fact suggests a secondary source of infection, either human to human transmission or possibly infected animals in another market in Wuhan. Both possibilities represent major concerns and indicate the outbreak has the potential to expand rapidly. For human to human transmission, there was limited data in the initial set of cases; one family cluster is of three men who all work in the market. Similarly, a husband and wife are among the patients, with the wife claiming no contact with the market. In these cases, direct human to human infection may have been possible; alternatively, a contaminated fomite from the market may also be responsible as surfaces all around the market were found to test positive 2019-nCoV. However, the major increase in the number of cases, the lack of direct connection to the Wuhan market for many cases, and the infection of health care works all suggest human to human spread is likely [9,44]. Importantly, until the source of the virus is found, it will be difficult to distinguish zoonotic versus human to human spread.

In the early part of the outbreak, the absence of infection in health care workers argued for inefficient human to human spread and distinguished 2019-nCoV from both SARS-CoV and MERS-CoV. In the two prior CoV epidemics, health care settings served as a major transmission point fueling both outbreaks. Based on WHO data, 1 in 10 MERS-CoV cases have been found to be health care workers; these patients generally have reduced disease and death likely due to younger age and absence of existing health conditions. The recent reports of numerous infected health care workers in Wuhan indicate human to human infection can occur with 2019-nCoV and may be the product of a super spreading patient [44]. However, while large swaths of healthcare workers are not getting sick as seen with SARS and MERS-CoV, it may be too early to rule out their potential exposure to the novel CoV as their disease may be asymptomatic. While not described during the SARS-CoV outbreak, asymptomatic cases ranged from 12.5% to 25% in some MERS-CoV studies [45]. A similar phenomenon may be occurring with 2019-nCoV and would make stopping the outbreak even more difficult to contain.

Another parameter to consider is the possibility of super spreading in the context of 2019-nCoV. Super spreading is the amplified transmission of a virus by individuals in a population and has been suggested by at least one news report [44]. Both SARS- and MERS-CoV outbreaks had documented evidence of super spreading patients [46]. In general, both epidemic CoVs maintain a low R_0, the rate spread from an individual infected patient. However, roughly 10% of SARS- and MERS-CoV patients have been associated with super spreading and an $R_0 > 10$. These cases seeded a significant portion of the epidemic around the world. Notably, neither mutations in the viruses nor severity of disease were found to be associated with super spreading, implying that host factors contribute to the phenotype [47]. For 2019-nCoV, contact tracing to date suggest limited human to human spread and a low R_0. However, the recent increase in cases, both in and outside Wuhan could signal the existence of super-spreading individuals fueling the outbreak. Alternatively, super spreading could occur from the zoonotic source which has been seen in other disease outbreaks [10]. In any event, the possibility of super spreading may continue to play a role in this ongoing 2019-nCoV outbreak.

7. Emerging Diseases in the Age of Social Media

News of the 2019-nCoV came to widespread attention through the internet. Over the years, websites like FluTrackers.com, ProMED (promedmail.org), and others have permitted the collection of disease information from around the world and facilitated dissemination to interested parties. In 2012, MERS-CoV first drew attention as a "novel coronavirus" entioned on ProMED Mail and subsequently through conversation on twitter between science journalists, virologists, and public health experts. Eight years later, a more connected network quickly dissected statements from the Wuhan Municipal Health Commission and speculated about possible causes. Early during an outbreak, it can be difficult to distinguish between rumors with elements of truth versus baseless fear mongering. This fact can be exacerbated by language barriers and off the record sources. However, in this case, speculation of a novel coronavirus was fed by carefully worded statements that specifically excluding some virus

families (influenza, adenovirus), but only excluded SARS-CoV and MERS-CoV for coronaviruses. Coupled with memories of the SARS outbreak, many worried that the truth may be held back. When the agent was finally confirmed as a CoV, the world acted with both worry and relief: the outbreak would not be hidden.

While far from perfect, the government response to 2019-nCoV provides a stark contrast to the SARS outbreak at the beginning of the century. The rapid release of 2019-nCoV sequences permitted the research community to quickly become engaged, providing analysis and developing diagnostic tests. Both the Chinese CDC and the Wuhan Municipal Health Commission have posted regular updates of confirmed case numbers and patient statuses enabling public health authorities to monitor the situation in real time. Researchers from around the world have connected on social media to compare updated sequence information and highlight key unknowns about the outbreak. While not always provided in a timely manner, the ability to share news updates and data in real time with researchers and public health officials around the world signals a major change in the response to outbreaks. This connectivity has facilitated awareness as well as new collaborations and a rapid response by the global research community. While there are many unknowns with 2019-nCoV, the world is engaged and prepared to battle the newest emergent virus strain. Perhaps this means the lessons from the SARS outbreak have truly been learned.

Author Contributions: L.E.G. and V.D.M. prepared this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wuhan Municipal Health Commision. Wuhan Municipal Health and Health Commission's Briefing on the Current Pneumonia Epidemic Situation in Our City. Available online: http://wjw.wuhan.gov.cn/front/web/showDetail/2019123108989 (accessed on 19 January 2020).
2. Kahn, N. New Virus Discovered by Chinese Scientists Investigating Pneumonia Outbreak. *Wall Street Journal*, 2020.
3. World Health Organization. WHO Statement Regarding Cluster of Pneumonia Cases in Wuhan, China. Available online: https://www.who.int/china/news/detail/09-01-2020-who-statement-regarding-cluster-of-pneumonia-cases-in-wuhan-china (accessed on 19 January 2020).
4. Virological.org. Novel 2019 Coronavirus Genome. Available online: http://virological.org/t/novel-2019-coronavirus-genome/319 (accessed on 19 January 2020).
5. GSAID Database. 2020 Coronavirus. Available online: https://www.gisaid.org/CoV2020/ (accessed on 19 January 2020).
6. World Health Organization. Novel Coronavirus—Japan (ex-China). Available online: https://www.who.int/csr/don/16-january-2020-novel-coronavirus-japan-ex-china/en/ (accessed on 19 January 2020).
7. World Health Organization. Laboratory Testing for 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases. Available online: https://www.who.int/health-topics/coronavirus/laboratory-diagnostics-for-novel-coronavirus (accessed on 19 January 2020).
8. Imai, N.; Dorigattig, I.; Cori, A.; Riley, S.; Ferguson, N.M. Estimating the Potential Total Number of Novel Coronavirus (2019-nCoV) Cases in Wuhan City, China. Available online: https://www.imperial.ac.uk/mrc-global-infectious-disease-analysis/news--wuhan-coronavirus/ (accessed on 19 January 2020).
9. Wuhan Municipal Health Commision. Wuhan Municipal Commission of Health and Health on Pneumonia of New Coronavirus Infection. Available online: http://wjw.wuhan.gov.cn/front/web/showDetail/2020012009077 (accessed on 20 January 2020).
10. Stein, R.A. Super-spreaders in infectious diseases. *Int. J. Infect. Dis.* **2011**, *15*, e510–e513. [CrossRef]
11. The Straits Times. Wuhan Virus: China Locks down Huanggang, Shuts Down Railway Station in Ezhou after Wuhan Lockdown. Available online: https://www.straitstimes.com/asia/east-asia/china-locks-down-two-more-cities-huanggang-and-ezhou-after-wuhan (accessed on 23 January 2020).

12. Ji, W.; Wang, W.; Zhao, X.; Zai, J.; Li, X. Homologous recombination within the spike glycoprotein of the newly identified coronavirus may boost cross-species transmission from snake to human. *J. Med. Virol.* **2020**. [CrossRef] [PubMed]
13. Robertson, D.; Jiang, X. nCoV's Relationship to Bat Coronaviruses and Recombination Signals No Snakes. Available online: http://virological.org/t/ncovs-relationship-to-bat-coronaviruses-recombination-signals-no-snakes/331 (accessed on 23 January 2020).
14. Kan, B.; Wang, M.; Jing, H.; Xu, H.; Jiang, X.; Yan, M.; Liang, W.; Zheng, H.; Wan, K.; Liu, Q.; et al. Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms. *J. Virol.* **2005**, *79*, 11892–11900. [CrossRef] [PubMed]
15. Lau, S.K.; Woo, P.C.; Li, K.S.; Huang, Y.; Tsoi, H.W.; Wong, B.H.; Wong, S.S.; Leung, S.Y.; Chan, K.H.; Yuen, K.Y. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 14040–14045. [CrossRef] [PubMed]
16. Menachery, V.D.; Yount, B.L., Jr.; Debbink, K.; Agnihothram, S.; Gralinski, L.E.; Plante, J.A.; Graham, R.L.; Scobey, T.; Ge, X.Y.; Donaldson, E.F.; et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat. Med.* **2015**, *21*, 1508–1513. [CrossRef]
17. Menachery, V.D.; Yount, B.L., Jr.; Sims, A.C.; Debbink, K.; Agnihothram, S.S.; Gralinski, L.E.; Graham, R.L.; Scobey, T.; Plante, J.A.; Royal, S.R.; et al. SARS-like WIV1-CoV poised for human emergence. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3048–3053. [CrossRef]
18. Wang, N.; Li, S.Y.; Yang, X.L.; Huang, H.M.; Zhang, Y.J.; Guo, H.; Luo, C.M.; Miller, M.; Zhu, G.; Chmura, A.A.; et al. Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. *Virol. Sin* **2018**, *33*, 104–107. [CrossRef]
19. Anthony, S.J.; Gilardi, K.; Menachery, V.D.; Goldstein, T.; Ssebide, B.; Mbabazi, R.; Navarrete-Macias, I.; Liang, E.; Wells, H.; Hicks, A.; et al. Further Evidence for Bats as the Evolutionary Source of Middle East Respiratory Syndrome Coronavirus. *mBio* **2017**, *8*. [CrossRef]
20. Quan, P.L.; Firth, C.; Street, C.; Henriquez, J.A.; Petrosov, A.; Tashmukhamedova, A.; Hutchison, S.K.; Egholm, M.; Osinubi, M.O.; Niezgoda, M.; et al. Identification of a severe acute respiratory syndrome coronavirus-like virus in a leaf-nosed bat in Nigeria. *mBio* **2010**, *1*. [CrossRef]
21. Azhar, E.I.; El-Kafrawy, S.A.; Farraj, S.A.; Hassan, A.M.; Al-Saeed, M.S.; Hashem, A.M.; Madani, T.A. Evidence for camel-to-human transmission of MERS coronavirus. *N. Engl. J. Med.* **2014**, *370*, 2499–2505. [CrossRef]
22. Fehr, A.R.; Channappanavar, R.; Perlman, S. Middle East Respiratory Syndrome: Emergence of a Pathogenic Human Coronavirus. *Annu. Rev. Med.* **2017**, *68*, 387–399. [CrossRef] [PubMed]
23. Hung, I.F.N.; Cheng, V.C.C.; Wu, A.K.L.; Tang, B.S.F.; Chan, K.H.; Chu, C.M.; Wong, M.M.L.; Hui, W.T.; Poon, L.L.M.; Tse, D.M.W.; et al. Viral Loads in Clinical Specimens and SARS Manifestations. *Emerg. Infect. Dis.* **2004**, *10*, 1550–1557. [CrossRef] [PubMed]
24. Assiri, A.; Al-Tawfiq, J.A.; Al-Rabeeah, A.A.; Al-Rabiah, F.A.; Al-Hajjar, S.; Al-Barrak, A.; Flemban, H.; Al-Nassir, W.N.; Balkhy, H.H.; Al-Hakeem, R.F.; et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: A descriptive study. *Lancet Infect. Dis.* **2013**, *13*, 752–761. [CrossRef]
25. Rahman, A.; Sarkar, A. Risk Factors for Fatal Middle East Respiratory Syndrome Coronavirus Infections in Saudi Arabia: Analysis of the WHO Line List, 2013–2018. *Am. J. Public Health* **2019**, *109*, 1288–1293. [CrossRef]
26. CIDRAP. SARS Severe Acute Respiratory Syndrome. Available online: http://www.cidrap.umn.edu/infectious-disease-topics/sars (accessed on 20 January 2020).
27. CIDRAP. MERS-CoV. Available online: http://www.cidrap.umn.edu/infectious-disease-topics/mers-cov (accessed on 20 January 2020).
28. Rambaut, A. Preliminary Phylogenetic Analysis of 11 nCoV2019 Genomes, 2020-01-19. Available online: http://virological.org/t/preliminary-phylogenetic-analysis-of-11-ncov2019-genomes-2020-01-19/329 (accessed on 20 January 2020).
29. Bedford, T.; Neher, R. Genomic Epidemiology of Novel Coronavirus (nCoV) Using Data Generated by Fudan University, China CDC, Chinese Academy of Medical Sciences, Chinese Academy of Sciences and the Thai National Institute of Health Shared via GISAID. Available online: https://nextstrain.org/ncov (accessed on 20 January 2020).

30. Shi, Z.-L.; Zhou, P.; Yang, X.-L.; Wang, X.-G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.-R.; Zhu, Y.; Li, B.; et al. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *bioRxiv* **2020**. [CrossRef]
31. Menachery, V.D.; Graham, R.L.; Baric, R.S. Jumping species-a mechanism for coronavirus persistence and survival. *Curr. Opin. Virol.* **2017**, *23*, 1–7. [CrossRef]
32. Becker, M.M.; Graham, R.L.; Donaldson, E.F.; Rockx, B.; Sims, A.C.; Sheahan, T.; Pickles, R.J.; Corti, D.; Johnston, R.E.; Baric, R.S.; et al. Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 19944–19949. [CrossRef]
33. Li, W.; Sui, J.; Huang, I.C.; Kuhn, J.H.; Radoshitzky, S.R.; Marasco, W.A.; Choe, H.; Farzan, M. The S proteins of human coronavirus NL63 and severe acute respiratory syndrome coronavirus bind overlapping regions of ACE2. *Virology* **2007**, *367*, 367–374. [CrossRef]
34. Zeng, L.P.; Gao, Y.T.; Ge, X.Y.; Zhang, Q.; Peng, C.; Yang, X.L.; Tan, B.; Chen, J.; Chmura, A.A.; Daszak, P.; et al. Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response. *J. Virol.* **2016**, *90*, 6573–6582. [CrossRef]
35. Xu, X.; Chen, P.; Wang, J.; Feng, J.; Zhou, H.; Li, X.; Zhong, W.; Hao, P. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci. China Life Sci.* **2020**. [CrossRef]
36. Letko, M.C.; Munster, V. Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV. *bioRxiv* **2020**. [CrossRef]
37. Rivers, T.M. Viruses and Koch's Postulates. *J. Bacteriol.* **1937**, *33*, 1–12. [CrossRef] [PubMed]
38. Roberts, A.; Deming, D.; Paddock, C.D.; Cheng, A.; Yount, B.; Vogel, L.; Herman, B.D.; Sheahan, T.; Heise, M.; Genrich, G.L.; et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathog.* **2007**, *3*, e5. [CrossRef] [PubMed]
39. McAuliffe, J.; Vogel, L.; Roberts, A.; Fahle, G.; Fischer, S.; Shieh, W.J.; Butler, E.; Zaki, S.; St Claire, M.; Murphy, B.; et al. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Virology* **2004**, *330*, 8–15. [CrossRef] [PubMed]
40. Kuiken, T.; Fouchier, R.A.; Schutten, M.; Rimmelzwaan, G.F.; van Amerongen, G.; van Riel, D.; Laman, J.D.; de Jong, T.; van Doornum, G.; Lim, W.; et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet (Lond. Engl.)* **2003**, *362*, 263–270. [CrossRef]
41. Van Doremalen, N.; Miazgowicz, K.L.; Milne-Price, S.; Bushmaker, T.; Robertson, S.; Scott, D.; Kinne, J.; McLellan, J.S.; Zhu, J.; Munster, V.J. Host species restriction of Middle East respiratory syndrome coronavirus through its receptor, dipeptidyl peptidase 4. *J. Virol.* **2014**, *88*, 9220–9232. [CrossRef]
42. Cockrell, A.S.; Peck, K.M.; Yount, B.L.; Agnihothram, S.S.; Scobey, T.; Curnes, N.R.; Baric, R.S.; Heise, M.T. Mouse dipeptidyl peptidase 4 is not a functional receptor for Middle East respiratory syndrome coronavirus infection. *J. Virol.* **2014**, *88*, 5195–5199. [CrossRef]
43. De Wit, E.; Rasmussen, A.L.; Falzarano, D.; Bushmaker, T.; Feldmann, F.; Brining, D.L.; Fischer, E.R.; Martellaro, C.; Okumura, A.; Chang, J.; et al. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 16598–16603. [CrossRef]
44. Wen, X. 15 Medical Staff in Wuhan Confirmed New Coronavirus Pneumonia, and Another Suspected. Available online: http://china.qianlong.com/2020/0121/3600877.shtml (accessed on 21 January 2020).
45. Al-Tawfiq, J.A.; Gautret, P. Asymptomatic Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection: Extent and implications for infection control: A systematic review. *Travel Med. Infect. Dis.* **2019**, *27*, 27–32. [CrossRef]
46. Wong, G.; Liu, W.; Liu, Y.; Zhou, B.; Bi, Y.; Gao, G.F. MERS, SARS, and Ebola: The Role of Super-Spreaders in Infectious Disease. *Cell Host Microbe* **2015**, *18*, 398–401. [CrossRef]
47. Park, D.; Huh, H.J.; Kim, Y.J.; Son, D.S.; Jeon, H.J.; Im, E.H.; Kim, J.W.; Lee, N.Y.; Kang, E.S.; Kang, C.I.; et al. Analysis of intrapatient heterogeneity uncovers the microevolution of Middle East respiratory syndrome coronavirus. *Cold Spring Harb. Mol. Case Stud.* **2016**, *2*, a001214. [CrossRef] [PubMed]

SARS-COV-2: salto di specie dai pipistrelli

Si stima che il 60% delle malattie infettive sia di origine animale, sebbene, in realtà, anche i patogeni attualmente ad esclusiva circolazione interumana anticamente è verosimile che abbiano compiuto un salto di specie (*spillover)*.
L'analisi dei genomi virali prelevati da campioni di pazienti cinesi nelle prime fasi dell'epidemia da nuovo coronavirus ha innanzitutto determinato una spiccata omologia (79,5%) con il virus responsabile della prima forma di SARS (*severe acute respiratory syndrome*), di cui si dimostrò l'origine dai pipistrelli: entrambi hanno quale target il recettore cellulare ACE2.

Altra analogia la sovrapposizione quasi identica (96%) con un coronavirus tipico dei chirotteri anche per il nuovo patogeno. Restano da capire nel dettaglio tempistiche e modalità dello spillover, che potrebbe avere coinvolto altri animali quali ospiti intermedi.

Riferimento bibliografico

Zhou P et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020 [Epub ahead of print].

Segue articolo full text originale

nature

https://doi.org/10.1038/s41586-020-2012-7

Accelerated Article Preview

A pneumonia outbreak associated with a new coronavirus of probable bat origin

Received: 20 January 2020

Accepted: 29 January 2020

Accelerated Article Preview
Published online 3 February 2020

Cite this article as: Zhou, P. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* https://doi.org/10.1038/s41586-020-2012-7 (2020).

Open access

Peng Zhou, Xing-Lou Yang, Xian-Guang Wang, Ben Hu, Lei Zhang, Wei Zhang, Hao-Rui Si, Yan Zhu, Bei Li, Chao-Lin Huang, Hui-Dong Chen, Jing Chen, Yun Luo, Hua Guo, Ren-Di Jiang, Mei-Qin Liu, Ying Chen, Xu-Rui Shen, Xi Wang, Xiao-Shuang Zheng, Kai Zhao, Quan-Jiao Chen, Fei Deng, Lin-Lin Liu, Bing Yan, Fa-Xian Zhan, Yan-Yi Wang, Geng-Fu Xiao & Zheng-Li Shi

Article

A pneumonia outbreak associated with a new coronavirus of probable bat origin

https://doi.org/10.1038/s41586-020-2012-7

Received: 20 January 2020

Accepted: 29 January 2020

Published online: 3 February 2020

Open access

Peng Zhou[1,5], Xing-Lou Yang[1,5], Xian-Guang Wang[2,5], Ben Hu[1], Lei Zhang[1], Wei Zhang[1], Hao-Rui Si[1,3], Yan Zhu[1], Bei Li[1], Chao-Lin Huang[2], Hui-Dong Chen[2], Jing Chen[1,3], Yun Luo[1,3], Hua Guo[1,3], Ren-Di Jiang[1,3], Mei-Qin Liu[1,3], Ying Chen[1,3], Xu-Rui Shen[1,3], Xi Wang[1,3], Xiao-Shuang Zheng[1,3], Kai Zhao[1,3], Quan-Jiao Chen[1], Fei Deng[1], Lin-Lin Liu[4], Bing Yan[1], Fa-Xian Zhan[4], Yan-Yi Wang[1], Geng-Fu Xiao[1] & Zheng-Li Shi[1]*

Since the SARS outbreak 18 years ago, a large number of severe acute respiratory syndrome-related coronaviruses (SARSr-CoV) have been discovered in their natural reservoir host, bats[1–4]. Previous studies indicated that some of those bat SARSr-CoVs have the potential to infect humans[5–7]. Here we report the identification and characterization of a novel coronavirus (2019-nCoV) which caused an epidemic of acute respiratory syndrome in humans in Wuhan, China. The epidemic, which started from 12 December 2019, has caused 2,050 laboratory-confirmed infections with 56 fatal cases by 26 January 2020. Full-length genome sequences were obtained from five patients at the early stage of the outbreak. They are almost identical to each other and share 79.5% sequence identify to SARS-CoV. Furthermore, it was found that 2019-nCoV is 96% identical at the whole-genome level to a bat coronavirus. The pairwise protein sequence analysis of seven conserved non-structural proteins show that this virus belongs to the species of SARSr-CoV. The 2019-nCoV virus was then isolated from the bronchoalveolar lavage fluid of a critically ill patient, which can be neutralized by sera from several patients. Importantly, we have confirmed that this novel CoV uses the same cell entry receptor, ACE2, as SARS-CoV.

Coronavirus has caused two large-scale pandemic in the last two decades, SARS and MERS (Middle East respiratory syndrome)[8,9]. It was generally believed that SARSr-CoV, mainly found in bats, might cause future disease outbreak[10,11]. Here we report on a series of unidentified pneumonia disease outbreaks in Wuhan, Hubei province, central China. Started from a local seafood market, the outbreak has grown substantial to infect 2050 people in China with 56 deaths and to infect 35 people in 11 other countries up to January 26, 2020[12]. Typical clinical symptoms of these patients are fever, dry cough, dyspnea, headache, and pneumonia. Disease onset may result in progressive respiratory failure due to alveolar damage (as observed by transverse chest CT images) and even death. The disease was determined as viral induced pneumonia by clinicians according to clinical symptoms and other criteria including body temperature rising, lymphocytes and white blood cells decreasing (sometimes normal for the later), new pulmonary infiltrates on chest radiography, and no obvious improvement upon three days antibiotics treatment. It appears most of the early cases had contact history with the original seafood market, but the disease progressed to human-to-human transmission now.

Samples from seven patients with severe pneumonia (six are seafood market sellers or delivers), who were enrolled in intensive unit cares at the beginning of the outbreak, were sent to WIV laboratory for pathogen diagnosis (Extended Data Table 1). As a CoV lab, we first used pan-CoV PCR primers to test these samples[13], considering the outbreak happened in winter and in a market, same environment as SARS. We found five PCR positive. A sample (WIV04) collected from bronchoalveolar lavage fluid (BALF) was analysed by metagenomics analysis using next-generation sequencing (NGS) to identify potential etiological agents. Of the 10,038,758 total reads, or 1582 total reads obtained after human genome filtering, 1378 (87.1%) matched sequences of SARSr-CoV (Fig. 1a). By *de novo* assembly and targeted PCR, we obtained a 29,891-bp CoV genome that shared 79.5% sequence identity to SARS-CoV BJ01 (GenBank accession number AY278488.2). High genome coverage was obtained by remapping the total reads to this genome (Extended Data Figure 1). This sequence has been submitted to GISAID (accession no. EPI_ISL_402124). Following the name by WHO, we tentatively call it novel coronavirus 2019 (2019-nCoV). Four more full-length genome sequences of 2019-nCoV (WIV02, WIV05, WIV06, and WIV07) (GISAID accession nos. EPI_ISL_402127-402130) that were above 99.9% identical to each other were subsequently obtained from other four patients using NGS and PCR (Extended Data Table 2).

The virus genome consists of six major open reading frames (ORFs) common to coronaviruses and a number of other accessory genes (Fig. 1b). Further analysis indicates that some of the 2019-nCoV genes shared less than 80% nt sequence identity to SARS-CoV. However, the seven conserved replicase domains in ORF1ab that were used for

[1]CAS Key Laboratory of Special Pathogens, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, People's Republic of China. [2]Wuhan Jinyintan Hospital, Wuhan, China. [3]University of Chinese Academy of Sciences, Beijing, People's Republic of China. [4]Hubei Provincial Center for Disease Control and Prevention, Wuhan, People's Republic of China. [5]These authors contributed equally: Peng Zhou, Xing-Lou Yang, Xian-Guang Wang. *e-mail: zlshi@wh.iov.cn

CoV species classification, are 94.6% aa sequence identical between 2019-nCoV and SARS-CoV, implying the two belong to same species (Extended Data Table 3).

We then found a short RdRp region from a bat coronavirus termed BatCoV RaTG13 which we previously detected in *Rhinolophus affinis* from Yunnan Province showed high sequence identity to 2019-nCoV. We did full-length sequencing to this RNA sample (GISAID accession no.EPI_ISL_402131). Simplot analysis showed that 2019-nCoV was highly similar throughout the genome to RaTG13 (Fig. 1c), with 96.2% overall genome sequence identity. Using the aligned genome sequences of 2019-nCoV, RaTG13, SARS-CoV and previously reported bat SARSr-CoVs, no evidence for recombination events was detected in the genome of 2019-nCoV. The phylogenetic analysis of full-length genome, RNA-dependent RNA polymerase (RdRp) gene and S gene sequences all showed that RaTG13 is the closest relative of the 2019-nCoV and form a distinct lineage from other SARSr-CoVs (Fig. 1d and Extended Data Figure 2). The receptor binding protein spike (S) gene was highly divergent to other CoVs (Extended Data Figure 2), with less than 75% nt sequence identity to all previously described SARSr-CoVs except a 93.1% nt identity to RaTG13 (Extended Data Table 3). The S genes of 2019-nCoV and RaTG13 S gene are longer than other SARSr-CoVs. The major differences in 2019-nCoV are the three short insertions in the N-terminal domain, and four out of five key residues changes in the receptor-binding motif, in comparison with SARS-CoV (Extended Data Figure 3). Whether the insertions in N-terminal domain of 2019-nCoV confers a sialic acid binding activity like MERS-CoV needs to be further studied. The close phylogenetic relationship to RaTG13 provides evidence for a bat origin of 2019-nCoV.

We rapidly developed a qPCR detection based on the receptor-binding domain of spike gene, the most variable region among genome (Fig. 1c). Our data show the primers could differentiate 2019-nCoV with all other human coronaviruses including bat SARSr-CoV WIV1, which is 95% identity to SARS-CoV (Extended Data Figure 4a and 4b). From the seven patients, we found 2019-nCoV positive in six BALF and five oral swab samples during the first sampling by qPCR and conventional PCR. However, we can no longer find viral positive in oral swabs, anal swabs, and blood from these patients during the second sampling (Fig. 2a). We have to point out that other qPCR targets including RdRp or E gene may be suggested for routine detection. Based on these findings, we presume that the disease should be transmitted through airway, yet we can't rule out other possibilities if the investigation extended to include more patients.

For serological detection of 2019-nCoV, we used previously developed bat SARSr-CoV Rp3 nucleocapsid protein (NP) as antigen in IgG and IgM ELISA test, which shared 92% amino acid identity to 2019-nCoV NP (Extended Data Figure 5) and showed no cross-reactivity against other human coronaviruses except SARSr-CoV[7]. As a research lab, we were only able to get five serum samples from the seven viral infected patients. We monitored viral antibody levels in one patient (ICU-06) at seven, eight, nine, and eighteen days after disease onset (Extended Data Table 2). A clear trend of IgG and IgM titre (decreased at the last day) increase was observed (Fig. 2b). For a second investigation, we tested viral antibody for five of the seven viral positive patients around twenty days after disease onset (Extended Data Table 1 and 2). All patient samples, but not samples from healthy people, showed strong viral IgG positive (Fig. 2b). We also found three IgM positive, indicating acute infection.

We then successfully isolated the virus (named 2019-nCoV BetaCoV/Wuhan/WIV04/2019), in both Vero and Huh7 cells using BALF sample from ICU-06 patient. Clear cytopathogenic effects were observed in cells after three days incubation (Extended Data Figure 6a and 6b). The identity of the strain WIV04 was verified in Vero E6 cells by immunofluorescence microscopy using cross-reactive viral NP antibody (Extended Data Figure 6c and 6d), and by metagenomic sequencing, from which most of the reads mapped to 2019-nCoV and qPCR showing viral load increase from day 1 to day 3 (Extended Data Figure 6e and 6f). Viral particles in ultrathin sections of infected cells displayed typical coronavirus morphology under electron microscopy (Extended Data Figure 6g). To further confirm the neutralization activity of the viral IgG positive samples, we conducted serum-neutralization assays in Vero E6 cells using the five IgG positive patient sera. We demonstrate that all samples were able to neutralize 120 TCID50 2019-nCoV at a dilution of 1:40-1:80. We also show that this virus could be cross-neutralized by horse anti-SARS-CoV serum (offered by L-F Wang) at dilutions 1:80, but the potential for cross reactivity with SARS-CoV antibodies needs to be confirmed with anti-SARS-CoV serum from humans (Extended Data Table 4).

Angiotensin converting enzyme II (ACE2) was known as cell receptor for SARS-CoV[14]. To determine whether 2019-nCoV also use ACE2 as a cellular entry receptor, we conducted virus infectivity studies using HeLa cells expressing or not expressing ACE2 proteins from humans, Chinese horseshoe bats, civet, pig, and mouse. We show that 2019-nCoV is able to use all but mouse ACE2 as an entry receptor in the ACE2-expressing cells, but not cells without ACE2, indicating which is likely the cell receptor of 2019-nCoV (Fig. 3). We also proved that 2019-nCoV does not use other coronavirus receptors, aminopeptidase N and dipeptidyl peptidase 4 (Extended Data Figure 7).

The study provides the first detailed report on 2019-nCoV, the likely etiology agent responsible for ongoing acute respiratory syndrome epidemic in Wuhan, central China. Viral specific nucleotide positive and viral protein seroconversion observed in all patients tested provides evidence of an association between the disease and the presence of this virus. However, there are still many urgent questions to be answered. The association between the 2019-nCoV and the disease has not been proved by animal experiments to full the Koch postulates. We don't know the transmission routine of this virus among hosts yet. It seems the virus is becoming more transmissible between human-to-human. We should closely monitor if the virus continue evolving to become more virulent. Owing to shortage of specific treatment and considering the relatedness between SARS-CoV and 2019-nCoV, some drugs and pre-clinical vaccine against SARS-CoV probably can be applied to this virus. Finally, considering the wide spread of SARSr-CoV in their natural reservoirs, future research should be focused on active surveillance of these viruses through a broader geographic regions. In the long-term, broad-spectrum antiviral drugs and vaccine should be prepared for the future emerging infectious diseases caused by this cluster of virus. Most importantly, strict regulations against the wildlife domestication and consuming should be implemented.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2012-7.

1. Li, W. D. *et al*. Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**, 676-679, (2005).
2. Ge, X. Y. *et al*. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-+, (2013).
3. Yang, L. *et al*. Novel SARS-like Betacoronaviruses in Bats, China, 2011. *Emerg Infect Dis* **19**, 989-991, (2013).
4. Hu, B. *et al*. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS pathogens* **13**, e1006698, (2017).
5. Menachery, V. D. *et al*. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513, (2015).
6. Menachery, V. D. *et al*. SARS-like WIV1-CoV poised for human emergence. *Proc Natl Acad Sci U S A* **113**, 3048-3053, (2016).
7. Wang, N. *et al*. Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. *Virol Sin* **33**, 104-107, (2018).
8. Drosten, C. *et al*. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New Engl J Med* **348**, 1967-1976, (2003).

9. Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. M. E. & Fouchier, R. A. M. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *New Engl J Med* **367**, 1814-1820, (2012).
10. Cui, J., Li, F. & Shi, Z. L. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* **17**, 181-192, (2019).
11. Fan, Y., Zhao, K., Shi, Z. L. & Zhou, P. Bat Coronaviruses in China. *Viruses* **11**, (2019).
12. Wuhan Municipal Health Commission. *Press statement related to novel coronavirus infection (in Chinese)*, 2020).
13. Poon, L. L. *et al*. Identification of a novel coronavirus in bats. *Journal of virology* **79**, 2001-2009, (2005).
14. Li, W. *et al*. Angiotensin-Converting Enzyme 2 Is a Functional Receptor for the Sars Coronavirus. *Nature* **426**, 450-454, (2003).

Article

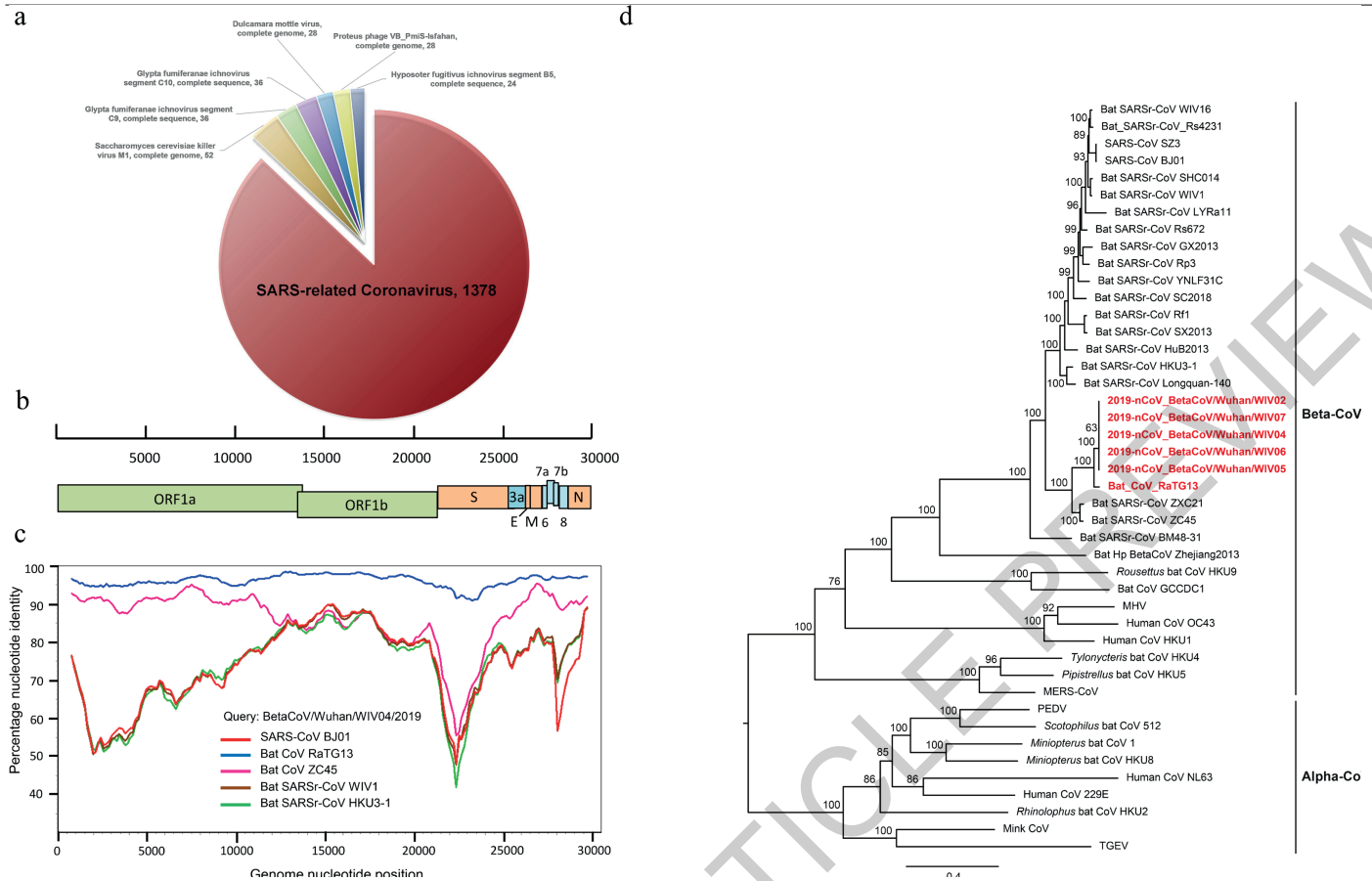


Fig. 1 | Genome characterization of 2019-nCoV. a, pie chart showing metagenomics analysis of next-generation sequencing of bronchoalveolar lavage fluid from patient ICU06. **b**, Genomic organization of 2019-nCoV WIV04. **c**, Similarity plot based on the full-length genome sequence of 2019-nCoV WIV04. Full-length genome sequences of SARS-CoV BJ01, bat SARSr-CoV WIV1, bat coronavirus RaTG13 and ZC45 were used as reference sequences. **d**, Phylogenetic tree based on nucleotide sequences of complete genomes of coronaviruses. Software used and settings can be found in material and method section.

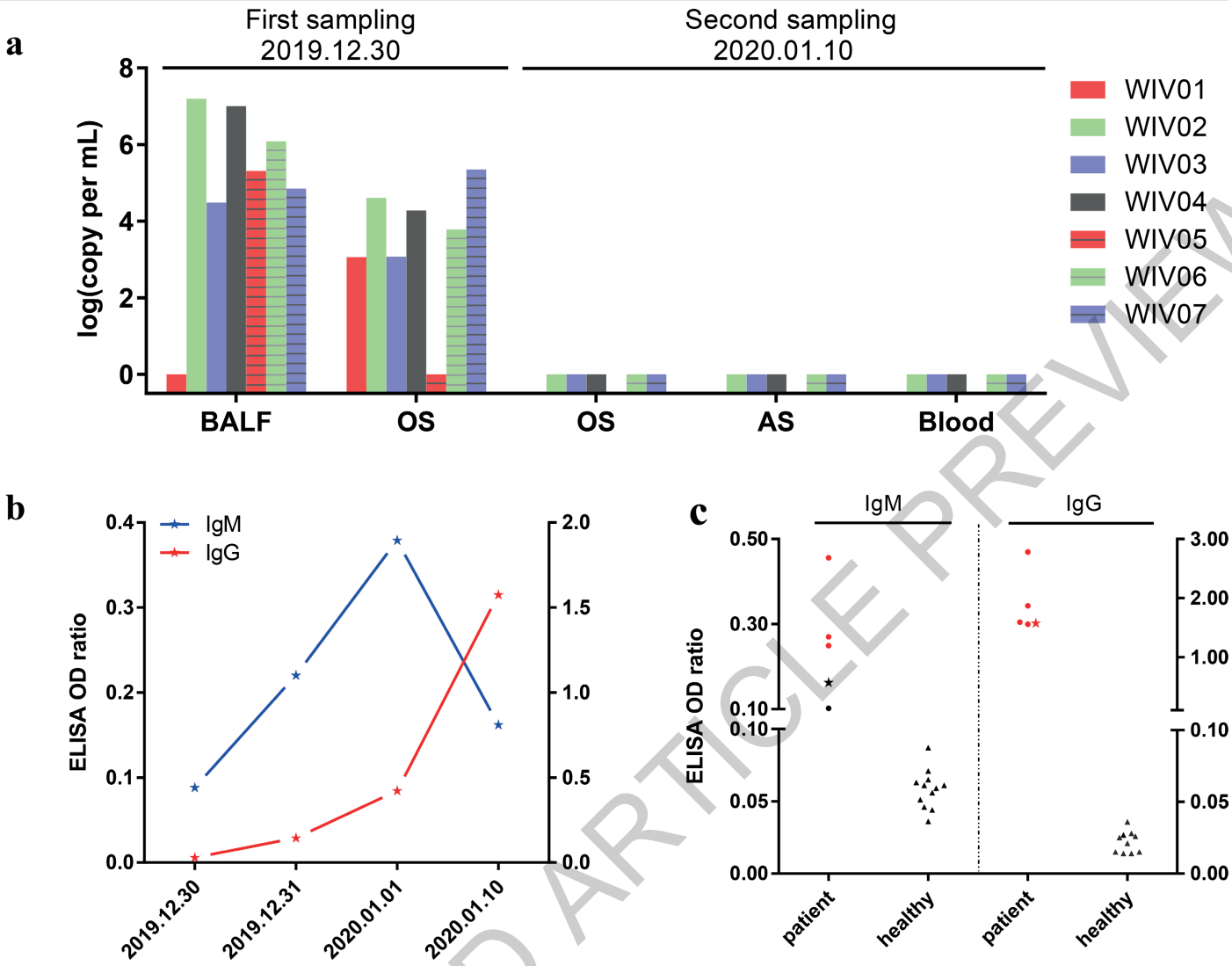


Fig. 2 | Molecular and serological investigation of patient samples. a, molecular detection of 2019-nCoV in seven patients during two times of sampling. Patient information can be found in Extended Data Table 1 and 2. Details on detection method can be found in material and methods. BALF, bronchoalveolar lavage fluid; OS, oral swab; AS, anal swab. **b**, dynamics of 2019-nCoV antibodies in one patient who showed sign of disease on 2019.12.23 (ICU-06). **c**, serological test of 2019-nCoV antibodies in five patients (more information can be found in Extended Data Table 2). Star indicates data collected from patient ICU-06 on 2020.01.10. For b and c, cut-off was set up as 0.2 for IgM test and 0.3 for IgG test, according to healthy controls.

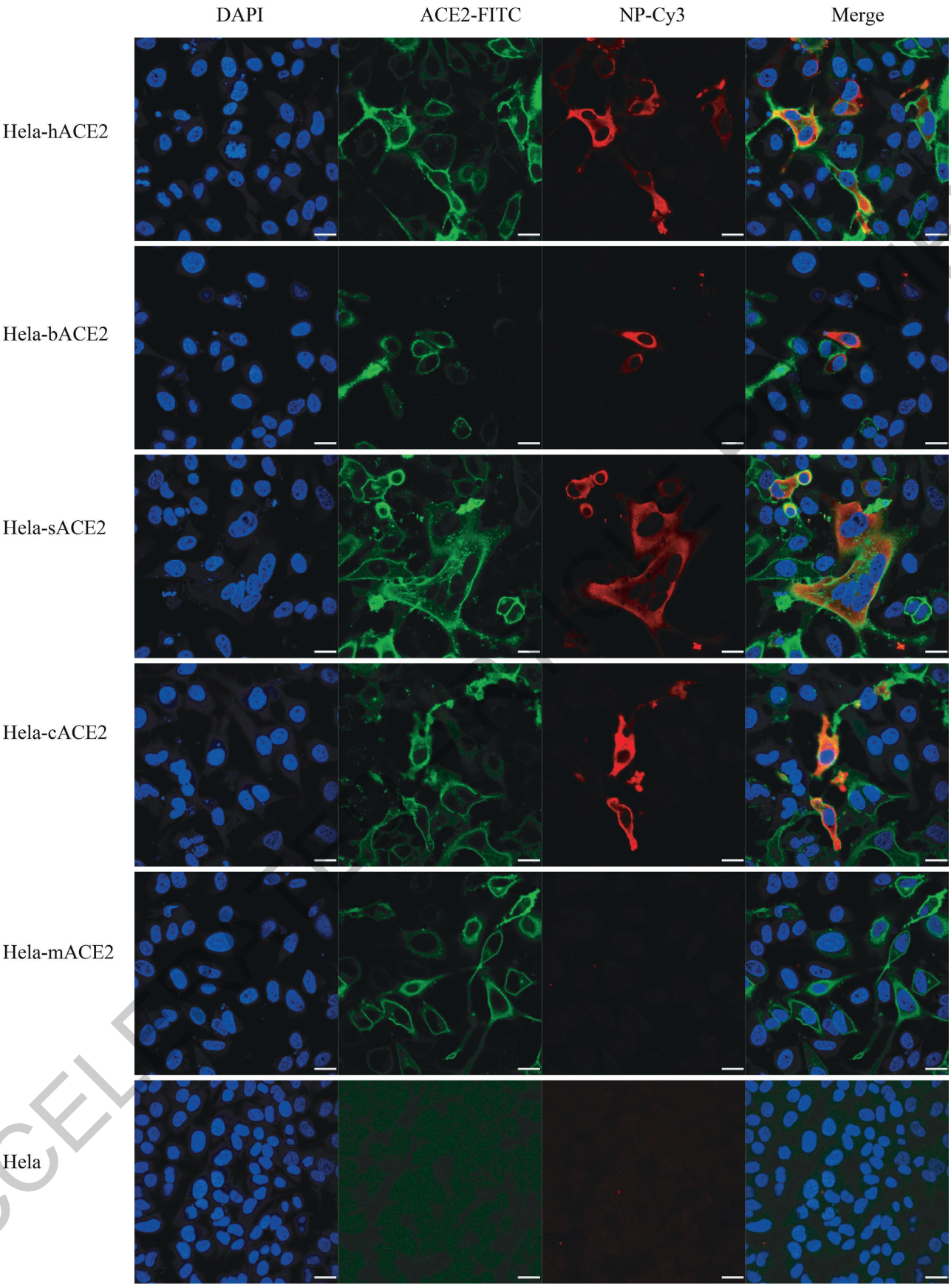


Fig. 3 | Analysis of 2019-nCoV receptor usage. Determination of virus infectivity in HeLa cells with or without the expression of ACE2. h, human; b, *Rhinolophus sinicus* bat; c, civet; s, swine (pig); m, mouse. ACE2 protein (green), viral protein (red) and nuclei (blue) was shown. Scale bar=10 um.

Methods

Sample collection

Human samples, including oral swabs, anal swabs, blood, and BALF samples were collected by Jinyintan hospital (Wuhan) with the consent from all patients and approved by the ethics commission of the designated hospital for emerging infectious diseases. Patients were sampled without gender or age preference unless where indicated. For swabs, 1.5 ml DMEM+2% FBS medium was added each tube. Supernatant was collected after 2500 rpm, 60 s vortex and 15-30 min standing. Supernatant from swabs or BALF (no pretreatment) was added to either lysis buffer for RNA extraction or to viral transport medium (VTM) for virus isolation. VTM composed of Hank's balanced salt solution at pH7.4 containing BSA (1%), amphotericin (15 μg/ml), penicillin G (100 units/ml), and streptomycin (50 μg/ml). Serum was separated by centrifugation at 3,000 *g* for 15 min within 24 h of collection, followed by 56 ^{0}C 30 min inactivation, and then stored at 4 ^{0}C until use.

Virus isolation, cell infection, electron microscope and neutralization assay

The following cells were used for virus isolation in this study: Vero, Vero E6, and Huh7 that were cultured in DMEM +10% FBS. A list of cells were used for susceptibility test (Extended Data Fig. 6). All cell lines were tested free of mycoplasma contamination, applied to species identification and authenticated by microscopic morphologic evaluation. None of cell lines was on the list of commonly misidentified cell lines (by ICLAC).

Cultured cell monolayers were maintained in their respective medium. PCR-positive BALF sample from ICU-06 patient was spin at 8,000 g for 15 min, filtered and diluted 1:2 with DMEM supplied with 16 μg/ml trypsin before adding to cells. After incubation at 37 ^{0}C for 1 h, the inoculum was removed and replaced with fresh culture medium containing antibiotics (below) and 16 μg/ml trypsin. The cells were incubated at 37 ^{0}C and observed daily for cytopathic effect (CPE). The culture supernatant was examined for presence of virus by qRT-PCR developed in this study, and cells were examined by immunofluorescent using SARSr-CoV Rp3 NP antibody made in house (1:100). Penicillin (100 units/ml) and streptomycin (15 μg/ml) were included in all tissue culture media.

The Vero E6 cells were infected with new virus at MOI of 0.5 and harvested 48 hpi. Cells were fixed with 2.5% (wt/vol) glutaraldehyde and 1% osmium tetroxide, and then dehydrated through a graded series of ethanol concentrations (from 30 to 100%), and embedded with epoxy resin. Ultrathin sections (80 nm) of embedded cells were prepared, deposited onto Formvar-coated copper grids (200 mesh), stained with uranyl acetate and lead citrate, then observed under 200 kV Tecnai G2 electron microscope.

The virus neutralization test was carried out in a 48-well plate. The patient serum samples were heat-inactivated by incubation at 56 ^{0}C for 30 min before use. The serum samples (5 μL) were diluted to 1:10, 1:20, 1:40 or 1:80, and then an equal volume of virus stock was added and incubated at 37 ^{0}C for 60 min in a 5% CO_2 incubator. Diluted horse anti SARS-CoV serum or serum samples from healthy people were used as control. After incubation, 100 μL mixtures were inoculated onto monolayer Vero E6 cells in a 48-well plate for 1 hour. Each serum were repeated triplicate. After removing the supernatant, the plate was washed twice with DMEM medium. Cells were incubated with DMEM supplemented with 2% FBS for 24 hours. Then the cells were fixed with 4% formaldehyde. And the virus were detected using SL-CoV Rp3 NP antibody followed by Cy3-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI. Infected cell number was counted by high-content cytometers.

RNA extraction and PCR

Whenever commercial kits were used, manufacturer's instructions were followed without modification. RNA was extracted from 200 μl of samples with the High Pure Viral RNA Kit (Roche). RNA was eluted in 50 μl of elution buffer and used as the template for RT-PCR.

For qPCR analysis, primers based on 2019-nCoV S gene was designed: RBD-qF1: 5′-CAATGGTTTAACAGGCACAGG-3′; RBD-qR1: 5′-CTCAAGTGTCTGTGGATCACG-3′. RNA extracted from above used in qPCR by HiScript® II One Step qRT-PCR SYBR® Green Kit (Vazyme Biotech Co.,Ltd). Conventional PCR test was also performed using the following primer pairs: ND-CoVs-951F TGTKAGRTTYCCTAAYATTAC; ND-CoVs-1805R ACATCYTGATANARAACAGC[13]. The 20 μl qPCR reaction mix contained 10 μl 2× One Step SYBR Green Mix, 1 μl One Step SYBR Green Enzyme Mix, 0.4 μl 50 × ROX Reference Dye 1, 0.4 μl of each primer (10 uM) and 2 μl template RNA. Amplification was performed as follows: 50 ^{0}C for 3 min, 95 ^{0}C for 30 s followed by 40 cycles consisting of 95 ^{0}C for 10 s, 60 ^{0}C for 30 s, and a default melting curve step in an ABI 7700 machine.

Serological test

In-house anti-SARSr-CoV IgG and IgM ELISA kits were developed using SARSr-CoV Rp3 NP as antigen, which shared above 90% amino acid identity to all SARSr-CoVs[2]. For IgG test, MaxiSorp Nunc-immuno 96 well ELISA plates were coated (100 ng/well) overnight with recombinant NP. Human sera were used at 1:20 dilution for 1 h at 37 ^{0}C. An anti-Human IgG-HRP conjugated monoclonal antibody (Kyab Biotech Co., Ltd, Wuhan, China) was used at a dilution of 1:40000. The OD value (450−630) was calculated. For IgM test, MaxiSorp Nunc-immuno 96 wellELISA plates were coated (500 ng/well) overnight with anti-human IgM (μ chain). Human sera were used at 1:100 dilution for 40 min at 37 ^{0}C, followed by anti-Rp3 NP-HRP conjugated (Kyab Biotech Co., Ltd, Wuhan, China) at a dilution of 1:4000. The OD value (450−630) was calculated.

Examination of ACE2 receptor for 2019-nCoV infection

HeLa cells transiently expressing ACE2 were prepared by a lipofectamine 3000 system (Thermo Fisher Scientific) in 96-well plate, with mock-transfected cells as controls. 2019-nCoV grown from Vero E6 cells was used for infection at multiplicity of infection 0.05. Same for testing of APN and DPP4. The inoculum was removed after 1 h absorption and washed twice with PBS and supplemented with medium. At 24 hpi, cells were washed with PBS and fixed with 4% formaldehyde in PBS (pH 7.4) for 20 min at room temperature. ACE2 expression was detected using mouse anti-S tag monoclonal antibody followed by FITC-labelled goat anti-mouse IgG H&L (Abcam, ab96879). Viral replication was detected using rabbit antibody against the Rp3 NP protein (made in house, 1:100) followed by cyanin 3-conjugated goat anti-rabbit IgG (1:50, Abcam, ab6939). Nucleus was stained with DAPI (Beyotime). Staining patterns were examined using the FV1200 confocal microscopy (Olympus).

High throughput sequencing, pathogen screening and genome assembly

Samples from patient BALF or from virus culture supernatant were used for RNA extraction and next-generation sequencing using BGI MGISEQ2000 and Illumina MiSeq 3000 sequencers. Metagenomic analysis was carried out mainly base on the bioinformatics platform MGmapper (PE_2.24 and SE_2.24). The raw NGS reads were firstly processed by Cutadapt (v1.18) with minimum read length of 30bp. BWA (v0.7.12-r1039) was utilized to align reads to local database with a filter hits parameter at 0.8 FMM value and minimum alignment score at 30. Parameters for post-processing of assigned reads was set with minimum size normalized abundance at 0.01, minimum read count at 20 and other default parameters. A local nucleic acid database for human and mammals was employed to filter reads of host genomes before mapping reads to virus database. The results of metagenomic analysis were displayed through pie charts using WPS Office 2010. NGS reads were assembled into genomes using Geneious (v11.0.3) and

MEGAHIT (v1.2.9). PCR and Sanger sequencing was performed to fill gaps in the genome. 5′-RACE was performed to determine the 5′-end of the genomes using SMARTer RACE 5′/3′ Kit (Takara). Genomes were annotated using Clone Manager Professional Suite 8 (Sci-Ed Software).

Phylogenetic analysis

Routine sequence management and analysis was carried out using DNAStar. The sequence alignment of complete genome sequences was performed by MAFFT (version 7.307) with default parameters. The codon alignments of full-length S and RdRp gene sequences were converted from the corresponding protein alignments by PAL2NAL (version 14), respectively, of which the protein alignments were created by Clustal Omega (version 1.2.4) under default parameters. Maximum Likelihood phylogenetic trees were carried out using RAxML (version 0.9.0) with GTR+G substitution model and 1000 bootstrap replicates.

Data availability

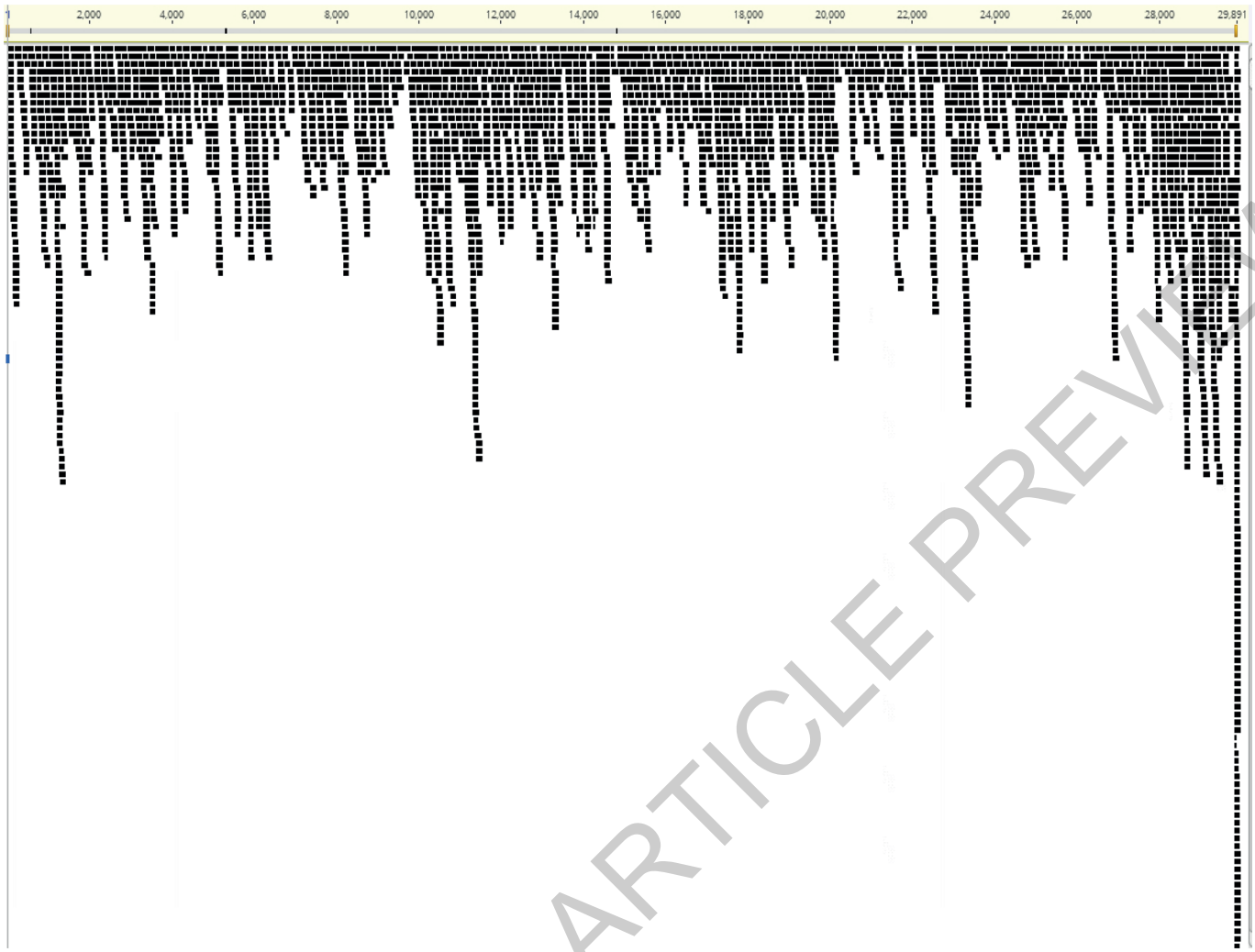
Sequence data that support the findings of this study have been deposited in GISAID with the accession numbers EPI_ISL_402124, EPI_ISL_402127–EPI_ISL_402130 and EPI_ISL_402131.

Acknowledgements We thank the Pei Zhang and An-na Du from WIV core facility and technical support for their help with producing TEM micrographs. We thank Hai-Zhou Liu and Ping Yu from WIV for bioinformatics analysis. This work was jointly supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB29010101 to ZLS and XDB29010104 to PZ), China Natural Science Foundation for excellent scholars (81822028 to PZ, 31770175 to ZLS and 31800142 to BH), Mega-Project for Infectious Disease from Minister of Science and Technology of the People's Republic of China (2020ZX09201001 to DYZ and 2018ZX10305409-004-001 to PZ), Youth innovation promotion association of CAS (2019328 to XLY).

Author contributions Z.L.S., P.Z., Y.Y.W., and G.F.X. conceived the study. G.S.W., C.L.H., H.D.C., F.D., Q.J.C., F.X.Z., and LLL., collected patient samples. X.L.Y., B.Y., W.Z., B.L., J.C., X.S.Z., Y.L., H.G., R.D.J., M.Q.L., Y. Chen, X.W., X.R.S., and K.Z. performed qPCR, serology, and virus culturing. L.Z., Y.Z., H.R.S., and B.H. performed genome sequencing and annotations.

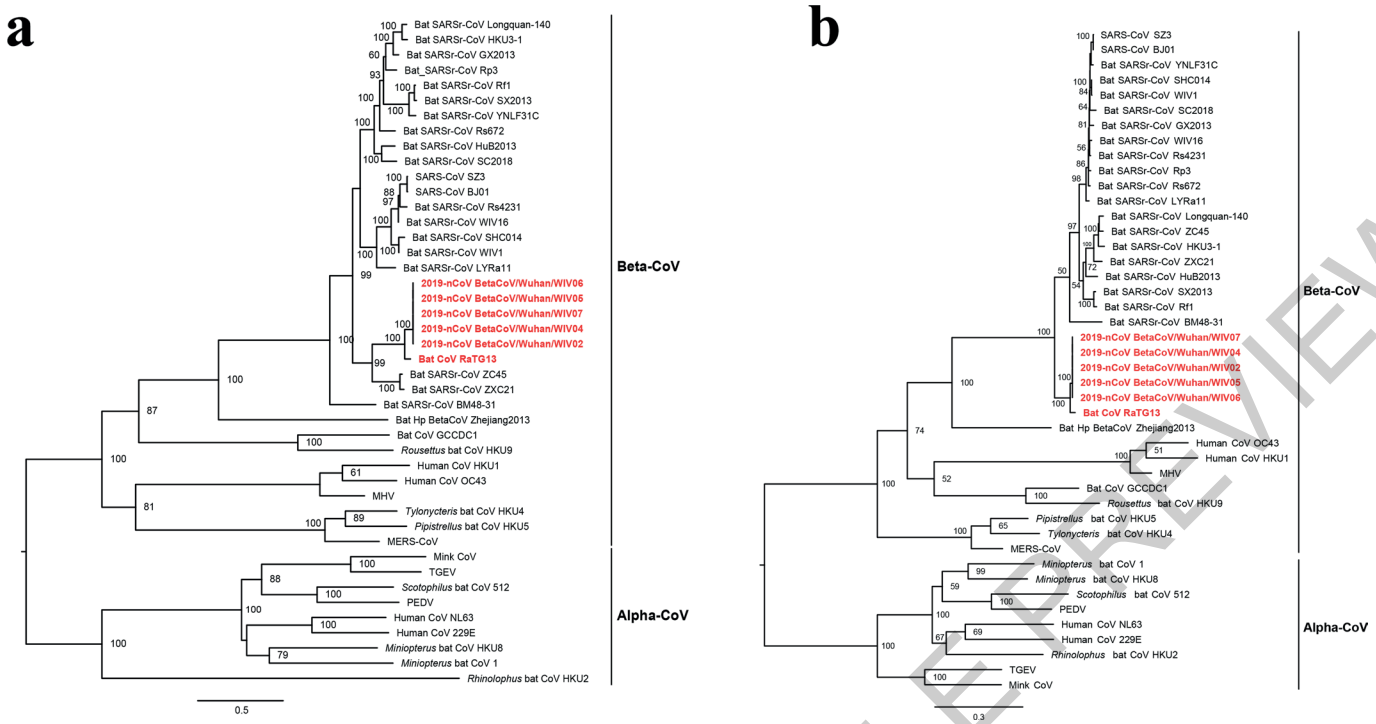
Competing interests The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to Z.-L.S.
Reprints and permissions information is available at http://www.nature.com/reprints.

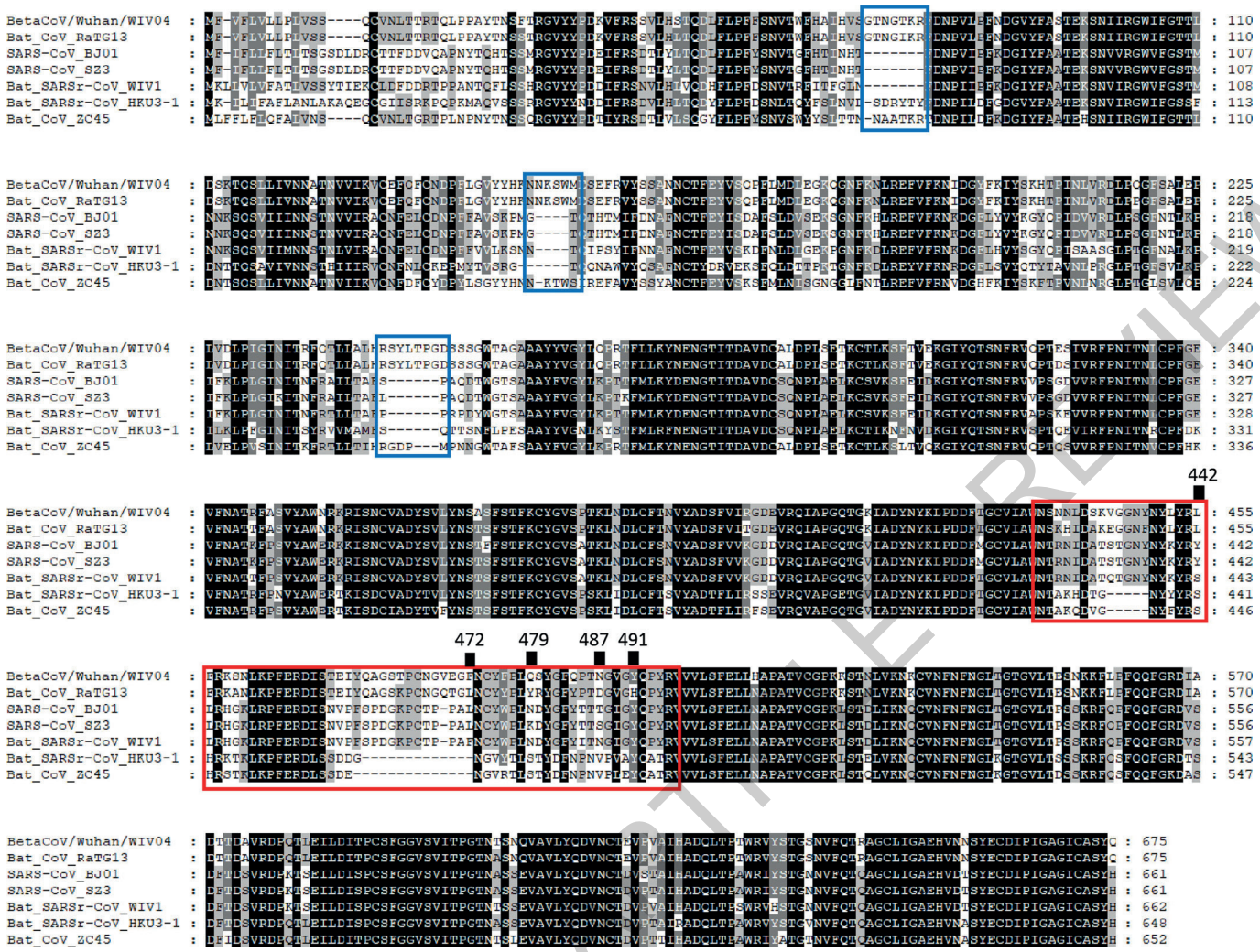


Extended Data Fig. 1 | NGS raw reads from WIV04 patient mapping to 2019-nCoV.

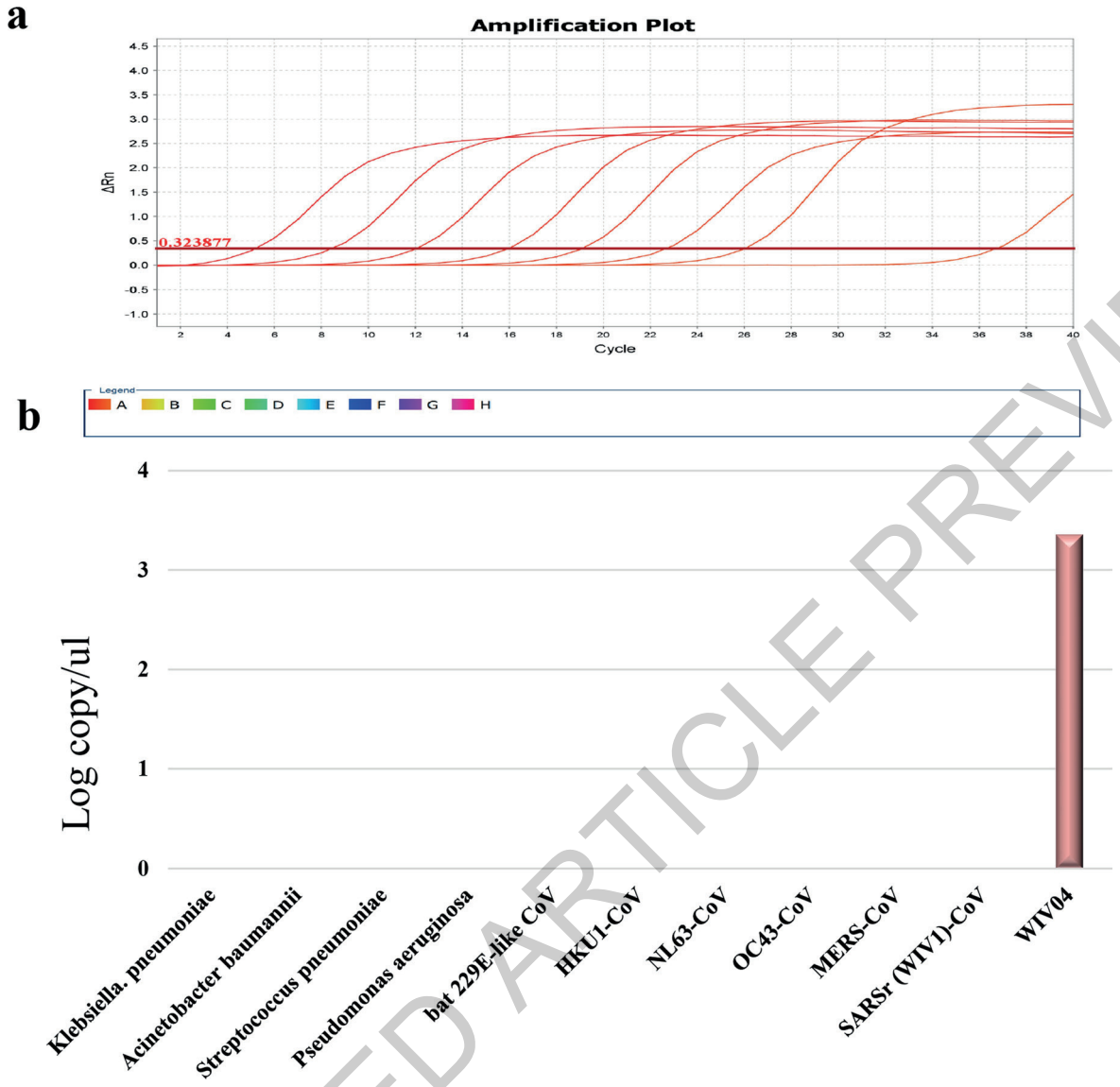
Article



Extended Data Fig. 2 | Phylogenetic tree base on the complete S (a) and RdRp (b) gene sequences of coronaviruses. 2019-nCoV and bat CoV RaTG13 are shown in bold and in red. The trees were constructed by the maximum likelihood method using the GTR+G substitution model with bootstrap values determined by 1000 replicates. Bootstraps > 50% are shown.

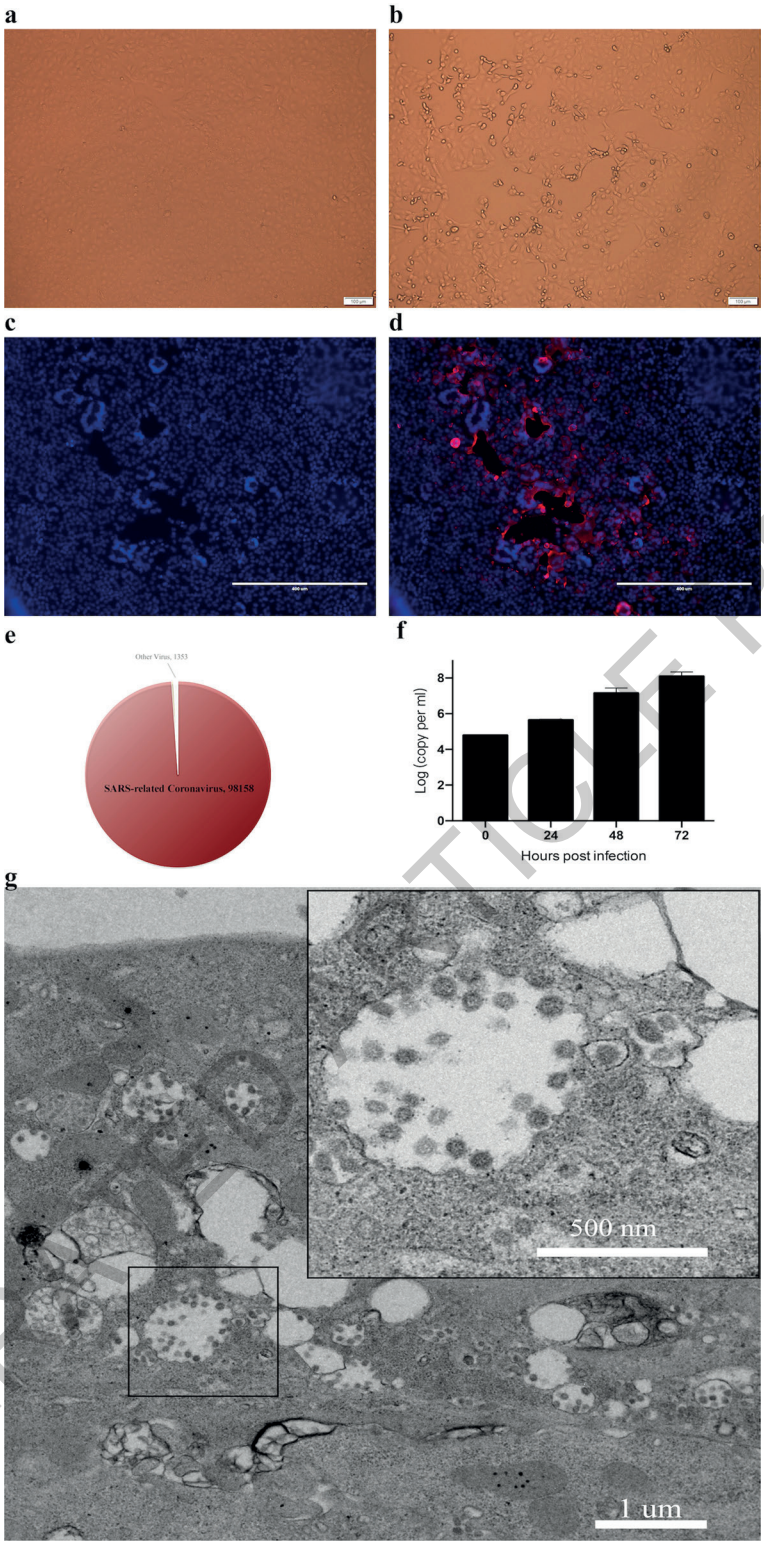


Extended Data Fig. 3 | Amino acid sequence alignment of the S1 protein of the 2019-nCoV with SARS-CoV and selected bat SARSr-CoVs. The receptor-binding motif of SARS-CoV and the homologous region of other coronaviruses are indicated by the red box. The key amino acid residues involved in the interaction with human ACE2 are numbered on top of the aligned sequences. The short insertions in the N-terminal domain of the novel coronavirus are indicated by the blue boxes. Bat CoV RaTG13 was identified from *R. affinis* in Yunnan Province. Bat CoV ZC45 was identified from *R. sinicus* in Zhejiang Province.

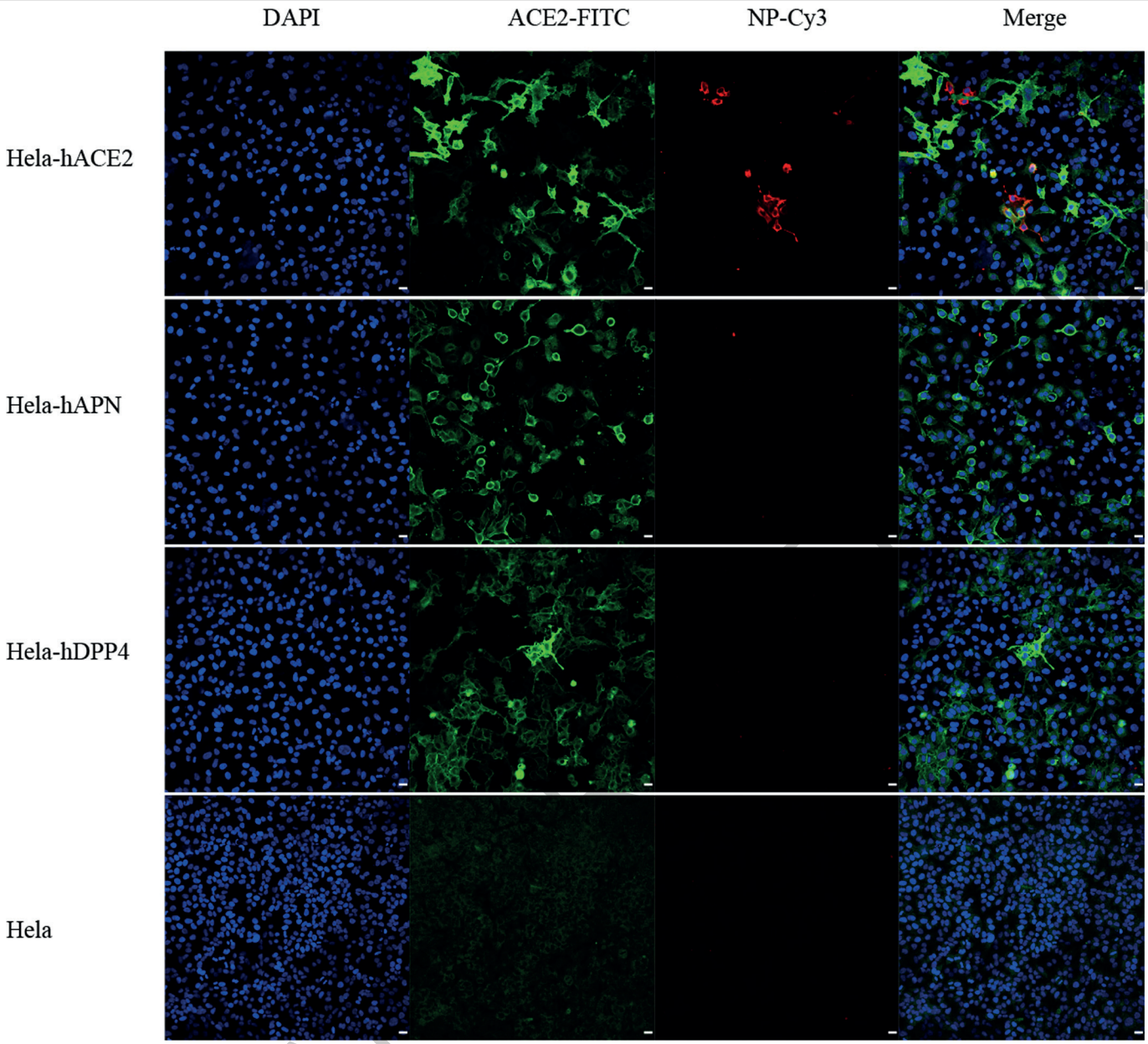


Extended Data Fig. 4 | Molecular detection method set up for 2019-nCoV. a, standard curve for qPCR primers. PCR product of spike gene that was serial diluted to 10^8 to 10^1 (from left to right) was used as template. Primer sequence and experiment condition can be found in material and methods. **b**, specificity of qPCR primers. Nucleotide samples from the indicated pathogens were used.

Extended Data Fig. 5 | Amino acid sequence alignment of the nucleocapsid protein of 2019-nCoV with bat SARSr-CoV Rp3 and SARS-CoV BJ01.



Extended Data Fig. 6 | Isolation and antigenic characterization of 2019-nCoV. Vero E6 cells are shown at 24 hours post infection with mock (**a**) or 2019-nCoV (**b**). (**c**) and (**d**) are mock or 2019-nCoV infected samples stained with rabbit serum raised against recombinant SARSr-CoV Rp3 N protein (red) and DAPI (blue). The experiment was conducted two times independently with similar results. **e** and **f**, pie charts illustrating ratio of reads number related to 2019-nCoV among total viral related reads in metagenomics analysis of Vero (**e**) and Huh7 (**f**) cell culture supernatant. (**g**) viral particles in the ultrathin sections under electron microscope at 200 kV, sample from viral infected Vero E6 cells.



Extended Data Fig. 7 | Analysis of 2019-nCoV receptor usage. Determination of virus infectivity in HeLa cells with or without the expression of human APN and DPP4. ACE2 protein (green), viral protein (red) and nuclei (blue) were shown. Scale bar=10 um.

Article

Extended Data Table 1 | Patient information and their diagnosis history (some records are missing)

Patient No.	Gender	Age	Date of Onset	Date of Admission	Symptoms When Admitted	Current Status (2020.01.13)	Diagnosis history
ICU-01*	Male	62	2019.12.12	2019.12.27	fever	recover, discharged	negative
ICU-04	Male	32	2019.12.19	2019.12.29	fever, cough, dyspnea	fever, intermittent cough	negative
ICU-05	Male	40	2019.12.17	2019.12.27	fever (38 °C), expectoration, malaise, dyspnea	fever, malaise, intermittent cough	AdV (IgM)
ICU-06	Female	49	2019.12.23	2019.12.27	fever (37.9 °C), palpitation	fever, malaise, cough	Coronavirus (nt)
ICU-08	Female	52	2019.12.22	2019.12.29	fever (38.5 °C), expectoration, malaise, dyspnea	recover, discharged	Streptococcus pneumoniae (nt)
ICU-09	Male	40	2019.12.22	2019.12.28	fever (38.5 °C), expectoration	fever (38.5 °C), malaise, expectoration, dizziness	negative
ICU-10	Male	56	2019.12.20	2019.12.20	fever, dyspnea, chest tightness	fever, malaise, cough, dyspnea	negative

All patients are seafood market sellers or deliverymen except ICU-01, whose contact history is unclear. All patients were in intensive care unit (ICU) during the first investigation, and now in stable condition. Blood IgM tests have been performed for the following respiratory pathogens for all patients: legionella pneumophilia, mycoplasma pneumoniae, chlamydia pneumoniae, respiratory syncytial virus, adenovirus, rickettsia, influenza A virus, influenza B virus, parainfluenza virus. *This patient reported fever on 2019.12.12, and then recovered without medical treatment. He came back to hospital on 2019.12.27 due to fever. His wife was also sick and admitted to hospital. Both of them were recovered.

Extended Data Table 2 | Laboratory detection results

		First sampling-2019.12.30			Second sampling-2020.01.10			
Patient No.	Test No.	BALF	Oral Swab	Blood (Ab)	Oral Swab	Anal Swab	Blood (PCR)	Blood (Ab)
ICU-01	WIV01	-	Ct=32.0	NA	NA	NA	NA	NA
ICU-04	WIV02[#]	Ct=17.6	Ct=26.6	NA	-	-	-	+
ICU-05	WIV03	Ct=27.0	Ct=31.9	NA	-	-	-	+
ICU-06	WIV04[#*]	Ct=18.3	Ct=27.7	+	-	-	-	+
ICU-08	WIV05[#]	Ct=24.1	-	NA	NA	NA	NA	NA
ICU-09	WIV06[#]	Ct=21.6	Ct=29.4	NA	-	-	-	+
ICU-10	WIV07[#]	Ct=25.7	Ct=24.0	NA	-	-	-	+

Samples from two patients (ICU-01 and ICU-08) were not available during the second investigation. They have been discharged from hospital. We did serial test for ICU-06 patient at the following date: 19.12.30, 19.12.31, 20.01.01 and 20.01.10, corresponding to seven, eight, nine and eighteen days upon disease onset (19.12.23). Table shows molecular and serological (IgM and IgG) detection results for 2019-nCoV. [$]Full-length genome obtained. *Virus isolated.

Article

Extended Data Table 3 | Genomic comparison of 2019-nCoV WIV04 with SARS-CoVs and bat SARSr-CoVs

		Sequence identities with SARS-CoVs & bat SARSr-CoVs (nt/aa %)									
	Full-length genome	ORF1a	ORF1b	S	ORF3a	E	M	ORF6	ORF7a	ORF7b	ORF8	N
SARS-CoV GZ02	79.6	76.0/80.9	86.2/95.7	73.4/77.0	75.6/73.4	94.7/96.0	85.4/90.5	76.3/68.9	82.8/86.0	84.8/81.4	52.0/31.6	87.7/91.2
SARS-CoV BJ01	79.6	76.0/80.8	86.2/95.7	73.4/76.9	75.3/72.6	94.7/96.0	85.6/90.5	75.8/67.2	82.8/86.0	84.8/81.4	51.1/-	88.8/91.2
SARS-CoV Tor2	79.6	76.0/80.9	86.2/95.8	73.4/76.7	75.4/72.6	94.7/96.0	85.6/90.5	76.3/68.9	82.8/86.0	84.8/81.4	51.1/-	88.8/91.2
SARS-CoV SZ3	79.6	76.0/81.0	86.2/95.8	73.4/76.9	75.4/72.6	94.7/96.0	85.3/90.0	76.3/68.9	82.8/86.0	84.8/81.4	52.3/31.6	88.8/91.2
SARS-CoV PC4-227	79.5	76.0/80.8	86.1/95.6	73.4/76.7	75.5/72.6	94.7/96.0	85.1/90.0	75.8/68.9	82.8/86.0	84.8/81.4	52.3/-	88.5/90.7
Bat SARr-CoV RaTG13	96.2	96.0/98.0	97.3/99.3	93.1/97.7	96.3/97.8	99.6/100	95.5/99.6	98.4/100	95.6/97.5	99.2/97.7	97.0/95.0	96.9/99.0
Bat SARr-CoV WIV1	79.7	76.0/80.7	85.9/95.8	73.4/77.6	76.1/74.5	95.6/96.0	84.8/90.0	78.0/73.8	85.0/88.4	85.6/83.7	65.8/57.9	88.5/90.9
Bat SARSr-CoV WIV16	79.7	75.9/81.0	86.1/95.6	73.1/77.8	76.1/74.5	95.6/96.0	84.8/90.0	77.4/72.1	85.0/88.4	85.6/83.7	65.3/57.9	88.6/90.9
Bat SARSr-CoV SHC014	79.6	75.9/80.9	85.9/95.8	73.3/77.7	76.1/74.5	95.6/96.0	84.8/90.0	78.0/70.5	84.4/88.4	85.6/83.7	65.8/58.7	88.6/90.9
Bat SARSr-CoV Rs4231	79.7	76.0/81.0	86.2/95.8	72.9/77.5	75.8/74.1	94.3/94.7	84.4/90.0	76.9/67.2	85.0/88.4	85.6/83.7	65.3/57.9	88.8/91.4
Bat SARSr-CoV YNLF31C	79.0	75.7/80.6	85.8/95.7	71.4/75.5	75.0/71.2	94.3/96.0	84.7/89.6	76.9/70.5	83.1/87.6	86.4/83.7	50.3/31.3	88.3/90.5
Bat SARSr-CoV LYRa11	79.6	75.8/80.6	85.7/95.6	73.9/77.3	77.2/76.3	94.7/94.7	85.1/90.0	78.5/70.5	82.0/85.1	81.1/81.4	66.7/57.9	89.0/91.6
Bat SARSr-CoV ZC45	88.1	91.0/95.7	86.1/96.0	77.8/82.3	87.8/90.9	98.7/100	93.4/98.6	95.2/93.4	88.8/87.6	94.7/93.0	88.5/94.2	91.1/94.3
Bat SARSr-CoV ZXC21	88.0	90.9/95.7	86.2/95.8	77.1/81.7	88.9/92.0	98.7/100	93.4/98.6	95.2/93.4	89.1/88.4	95.5/93.0	88.5/94.2	91.2/94.3
Bat SARSr-CoV HuB2013	79.6	76.3/81.2	85.3/95.7	73.1/76.8	75.4/75.5	95.2/94.7	85.3/91.0	76.3/68.9	84.2/87.6	85.6/83.7	62.0/49.6	88.9/91.6
Bat SARSr-CoV GX2013	79.1	75.9/80.8	86.0/95.9	73.1/77.1	75.6/73.0	94.7/96.0	84.8/91.4	77.4/68.9	85.0/86.8	84.1/79.1	51.4/31.6	87.9/90.2
Bat SARSr-CoV SX2013	78.9	76.2/80.6	85.1/95.5	71.2/75.5	74.7/71.2	94.3/93.3	83.0/89.6	77.4/68.9	84.2/86.8	85.6/83.7	49.7/30.4	86.9/90.2
Bat SARSr-CoV SC2018	79.4	75.8/80.7	85.5/95.2	72.7/76.4	75.0/71.2	94.3/96.0	84.7/90.0	80.0/71.8	85.2/87.6	84.8/83.7	66.1/55.4	88.2/91.2
Bat SARSr-CoV Rs672	79.6	76.0/80.9	85.9/95.8	72.8/76.2	75.2/71.9	95.2/96.0	84.8/89.6	78.5/70.5	84.7/88.4	85.6/83.7	65.8/58.7	87.9/91.2
Bat SARSr-CoV Rp3	79.5	75.9/80.5	86.0/95.7	73.1/77.2	74.9/74.8	95.2/96.0	85.1/90.0	76.9/68.9	83.9/89.3	84.8/83.7	66.4/56.2	88.4/90.7
Bat SARSr-CoV Rf1	78.8	76.2/80.6	84.8/95.3	71.1/75.7	74.3/69.0	94.3/94.7	83.3/89.6	79.0/68.9	84.2/86.8	84.1/83.7	50.6/31.3	86.8/89.5
Bat SARSr-CoV HKU3-1	79.4	76.1/80.9	84.9/95.1	73.4/77.9	75.8/73.4	95.2/96.0	84.7/91.0	75.3/67.2	85.0/89.3	84.1/79.1	66.4/57.0	88.3/90.0

Extended Data Table 4 | Virus neutralization test (VNT) of serum samples

Samples	VNT titre for nCoV-2019
Healthy people #1 from Wuhan	neg
Healthy people #2 from Wuhan	neg
Horse anti-SARS-CoV serum	>1:80
WIV02	>1:80
WIV03	1:40
WIV04	>1:80
WIV06	>1:80
WIV07	>1:80

Each serum sample was tested in triplicate. Two healthy people from Wuhan, five patient serum samples and a horse anti-SARS-CoV anti-serum were used. 120 $TCID_{50}$ viruses were used each well. Serum samples were used in a dilution from 1:10, 1:20, 1:40 to 1:80.

nature research

Corresponding author(s): Zheng-Li Shi

Last updated by author(s): Jan 26, 2020

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used.
Data analysis	BWA (v0.712-r1039), Cutadapt (v1.18), Geneious (v11.0.3), MEGAHIT (v1.2.9), Clone Manager Professional Suite 8, MAFFT (v7.307), MGmapper (PE2.24 and SE2.24), PAL2NAL (version 14), Clustal Omega (version 1.2.4), RAxML (version 0.9.0)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequence data that support the findings of this study have been deposited in GISAID with the accession no. EPI_ISL_402124 and EPI_ISL_402127-402130.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Samples of seven pneumonia patients are available from the clinical hospital to be sent to Wuhan Institute of Virology for pathogen identification. The coronavirus genome sequences were obtained from 5 different patients and shared >99.9% identity, suggesting they were infected by the same virus. Therefore, the sample size is sufficient for conducting the following study which aims to identify and characterize the causative agent of this pneumonia outbreak.
Data exclusions	No data excluded
Replication	The authors guarantee the findings are reliably reproducible. At least three independent experiments were performed, which was stated in the text.
Randomization	Samples were chosen randomly.
Blinding	We were blinded when choosing samples.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	1. SARSr-CoV Rp3 NP antibody made in house; 2. Cy3-conjugated mouse anti-rabbit IgG; 3. Anti-Human IgG-HRP conjugated monoclonal antibody (Kyab Biotech Co., Ltd, Wuhan, China, dilution: 1:40000); 4. Anti-Rp3 NP-HRP conjugated (Kyab Biotech Co., Ltd, Wuhan, China, dilution: 1:4000); 5. FITC-labelled goat anti-mouse IgG H&L (Abcam, ab96879, dlilution 1:100); 6. cyanin 3-conjugated goat anti-rabbit IgG (Abcam,ab6939, dilution: 1:50); 7. mouse anti-S tag monoclonal antibody made in house (1:10000)
Validation	The house-made SARSr-CoV Rp3 NP antibodies and anti-S tag monoclonal antibody were validated in a WB. The cy3-conjugated anti-rabbit IgGs were validated in IFA. The FITC-labelled goat anti-mouse IgG H&L was validated in IHC.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	1. African green monkey origin, Vero and Vero E6 cells; 2. Human lung cell Huh7 ; 3. Human HeLa cells. All cell lines were from ATCC.
Authentication	All monkey and human cells were from ATCC with authentication. The authentication was performed by microscope morphology check, growth curve analysis or identity verification with STR analysis (for human cell lines).
Mycoplasma contamination	We confirm that all cells were tested as mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Participants were all 2019-nCoV infected patients.
Recruitment	Samples were sent to Wuhan Institute of Virology by hospital for pathogen identification.
Ethics oversight	Wuhan Jinyintan Hospital (the co-authored institution)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

COVID-19: la prima linea guida

La gestione di un focolaio epidemico ad alta contagiosità nelle sue fasi iniziali è sempre difficile. La complessità aumenta nel caso di un nuovo patogeno, come SARS-COV-2.

Partendo tuttavia dalle esperienze maturate con the SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome) e influenza, un gruppo di ricercatori cinesi dell'ospedale universitario di Zhongnan (Wuhan) ha approntato una rapida linea guida per la gestione dell'infezione da SARS-COV-2.

Raccogliendo tutte le evidenze disponibili fino al termine di gennaio 2020 e seguendo le regole stabilite dall'Organizzazione Mondiale della Sanità per lo sviluppo di linee guida, il gruppo di lavoro cinese, forte anche dell'esperienza sul campo, ha elaborato il primo documento per la diagnosi e la gestione dei casi di COVID-19.

Riferimento bibliografico

Jin JH P et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res. 2020; 7: 4.

Segue articolo full text originale

Jin et al. Military Medical Research (2020) 7:4
https://doi.org/10.1186/s40779-020-0233-6

MMR MILITARY MEDICAL RESEARCH

POSITION ARTICLE AND GUIDELINE Open Access

A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version)

Ying-Hui Jin[1], Lin Cai[2], Zhen-Shun Cheng[3], Hong Cheng[4], Tong Deng[1,5], Yi-Pin Fan[6,7], Cheng Fang[1], Di Huang[1], Lu-Qi Huang[6,7], Qiao Huang[1], Yong Han[2], Bo Hu[8], Fen Hu[8], Bing-Hui Li[1,5], Yi-Rong Li[9], Ke Liang[10], Li-Kai Lin[2], Li-Sha Luo[1], Jing Ma[8], Lin-Lu Ma[1], Zhi-Yong Peng[8], Yun-Bao Pan[9], Zhen-Yu Pan[11], Xue-Qun Ren[5], Hui-Min Sun[12], Ying Wang[13], Yun-Yun Wang[1], Hong Weng[1], Chao-Jie Wei[3], Dong-Fang Wu[4], Jian Xia[14], Yong Xiong[10], Hai-Bo Xu[15], Xiao-Mei Yao[16], Yu-Feng Yuan[2], Tai-Sheng Ye[17], Xiao-Chun Zhang[15], Ying-Wen Zhang[17], Yin-Gao Zhang[2], Hua-Min Zhang[6,7], Yan Zhao[14], Ming-Juan Zhao[1], Hao Zi[1,5], Xian-Tao Zeng[1,18*], Yong-Yan Wang[6,7*], Xing-Huan Wang[1,2*], for the Zhongnan Hospital of Wuhan University Novel Coronavirus Management and Research Team, Evidence-Based Medicine Chapter of China International Exchange and Promotive Association for Medical and Health Care (CPAM)

Abstract

In December 2019, a new type viral pneumonia cases occurred in Wuhan, Hubei Province; and then named "2019 novel coronavirus (2019-nCoV)" by the World Health Organization (WHO) on 12 January 2020. For it is a never been experienced respiratory disease before and with infection ability widely and quickly, it attracted the world's attention but without treatment and control manual. For the request from frontline clinicians and public health professionals of 2019-nCoV infected pneumonia management, an evidence-based guideline urgently needs to be developed. Therefore, we drafted this guideline according to the rapid advice guidelines methodology and general rules of WHO guideline development; we also added the first-hand management data of Zhongnan Hospital of Wuhan University. This guideline includes the guideline methodology, epidemiological characteristics, disease screening and population prevention, diagnosis, treatment and control (including traditional Chinese Medicine), nosocomial infection prevention and control, and disease nursing of the 2019-nCoV. Moreover, we also provide a whole process of a successful treatment case of the severe 2019-nCoV infected pneumonia and experience and lessons of hospital rescue for 2019-nCoV infections. This rapid advice guideline is suitable for the first frontline doctors and nurses, managers of hospitals and healthcare sections, community residents, public health persons, relevant researchers, and all person who are interested in the 2019-nCoV.

Keywords: 2019 novel coronavirus, 2019-nCoV, Respiratory disease, Pneumonia, Infectious diseases, Rapid advice guideline, Clinical practice guideline, Evidence-based medicine

* Correspondence: zengxiantao1128@163.com; wangyyanpublic@bta.net.cn; wangxinghuan1965@163.com
Except the first and corresponding authors, the rest of authors were alphabetized by their last name and contributed equally.
[1]Center for Evidence-Based and Translational Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, China
[6]China Academy of Chinese Medical Sciences, Beijing 100700, China
Full list of author information is available at the end of the article

1 Background

In December 2019, the 2019 novel coronavirus (2019-nCoV) was discovered and identified in the viral pneumonia cases that occurred in Wuhan, Hubei Province, China; And then was named by the World Health Organization (WHO) on 12 January 2020. In the following month, the 2019-nCoV quickly spreading inside and outside of Hubei Province and even other countries. What's more, the sharp increase of the case number caused widespread panic among the people.

Medical professionals require an up-to-date guideline to follow when an urgent healthcare problem emerging. In response to the requests for reliable advice from frontline clinicians and public healthcare professionals managing 2019-nCoV pandemics, we developed this rapid advance guideline, involving disease epidemiology, etiology, diagnosis, treatment, nursing, and hospital infection control for clinicians, and also for public health workers and community residents.

2 Guideline methodology

This guideline was prepared in accordance with the methodology and general rules of WHO Guideline Development and the WHO Rapid Advice Guidelines [1, 2].

2.1 Composition of the guideline development group

This guideline development group is multidisciplinary and composed of individuals from health professionals and methodologists. Health professionals included frontline clinical doctors, nurses who work in departments of respiratory medicine, fever clinic, critical medicine, emergency, infectious disease, and experts of respiratory infectious disease and hospital management board. The methodologists included methodologists of guideline development, systematic review, and literature searching professionals.

2.2 The end-user of the guideline

This guideline is suitable for frontline doctors and nurses, managers of hospitals and healthcare sections, healthy community residents, personnel in public healthcare, relevant researchers, and all persons who are interested in the 2019-nCoV management.

2.3 The target population of the guideline

This guideline is aimed to serve the healthcare professionals to tackle the suspected 2019-nCoV infected cases, confirmed 2019-nCoV infected cases, clustered 2019-nCoV infected cases, and those with close contacts or suspicious exposure to 2019-nCoV infected cases.

2.4 A survey of conflict of interests

Oral inquiry for financial interests of relevant personal was conducted at the first meeting while to start this guideline. Relevant financial as well as nonfinancial interests were surveyed and disclosed and subsequently assessed in consensus conference in order to minimize potential bias in guideline development. Finally, there is no conflict of interests for all the personnel participating to prepare this guideline.

2.5 Guideline's structural setup and refining the topics and coverage of this guideline

This guideline is a rapid guideline to responding to the emerging infectious disease of 2019-nCoV. Due to the urgent need and tight work schedule, we conducted no wide-range survey but a discussion meeting with front-line clinicians who managed patients with 2019-nCoV infections to finalize guideline topics and key questions.

2.6 Literature searching and preparation of evidence profiles

2.6.1 General notes

Considering the lack of direct evidence for this newly identified 2019-nCoV infection, we searched and referred to the guidelines that related to the SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome), and influenza. We also referred to the guidelines which were newly-issued by the National Health Commission of People's Republic of China and WHO for 2019-nCoV. In addition, we have an independent literature searching team to search available indirect evidence from systematic reviews and/or RCTs (randomized controlled trials), which were for the treatment and/ or chemoprophylaxis of SARS, MERS, or other influenza virus infections.

If the existing evidence addressed topics or questions covered by the guideline, then its quality should be assessed. If there is a lack of higher-level quality evidence, our panel considered observational studies and case series. Because of the limited time, we did not perform new systematic review. We identified relevant literature up to 20 January 2020.

2.6.2 Search resources

We searched the bibliographic databases: PubMed, Embase, and Cochrane library.

We also searched following websites: the WHO (https://www.who.int/), CDC (Centers for Disease Control and Prevention, https://www.cdc.gov/), NICE (National Institute for Health and Clinical Excellence, https://www.nice.org.uk/), National Health Commission of the People's Republic of China (http://www.nhc.gov.cn/), and National Administration of Traditional Chinese Medicine (http://www.satcm.gov.cn/).

2.6.3 Frontline data collection and summary

As the 2019-nCoV is a newly identified pathogen responsible for the outbreak of the pandemic disease, there is no sufficient evidence to reveal the whole nature of this virus. In these situations, obtaining evidence from the experts who fighting the disease on the frontline can be efficient and the main source [3].

Until to 24:00 on 29 January 2020, 11,500 persons were screened, and 276 were identified as suspected infectious victims, and 170 were diagnosed (including 33 in critical condition) for 2019-nCoV infection in Zhongnan Hospital of Wuhan University. During this process, frontline clinicians and nurses have accumulated valuable experience in the diagnosis, treatment and nursing for 2019-nCoV infected patients. Hence, these experiences were assessed and then used as "Expert Evidence" for our guideline development. We took interviews and group surveys to collect information on treatment evidence during the guideline panel's meeting, so that it could be integrated into the guideline panel in the summary of findings (see Additional files 1 and 2). Experts' evidence can be solicited by the description of case reports, summaries, and reports of topics or questions of all cases they management.

2.7 Grading the evidences and recommendations

We accorded to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) basic approaches and rules [4, 5], and particularly considered experts' evidence to assess the quality of a body of evidence to make recommendations.

The quality of evidence reflects whether the extent to which our confidence estimating the effect is adequate to support a particular recommendation. The level of evidence was categorized as "high quality", "moderate quality", "low quality", or "very low quality"; Recommendations were classified as "strong" or "weak."

The strong recommendation does not always mean there is sufficient intervention effectiveness. Besides the effectiveness of intervention, the forming of recommendations is based on the severity of the disease, patient willingness, safety, and economics [4]. See Tables 1 and 2 [4, 6].

Table 1 Classification and description of recommendation

Classification of recommendation	Description
Strong recommendation	It is definite that the desirable effects of an intervention outweigh its undesirable effects or the undesirable effects of an intervention outweigh its desirable effects
Weak recommendation	The desirable effects probably outweigh the undesirable effects or undesirable effects probably outweigh the desirable effects

2.8 Forming the recommendations

Before meetings, experts' evidence was collected systematically and available to panel members. Once the evidence has been identified and assessed, recommendations were formulated based on the evidence by a face-to-face meeting of panel members and supplemented by experts participating in the panel meeting.

Experts' evidence was highly valued in this guideline development. During the consensus process, if the evidence was agreed on by more than 70% frontline clinicians in the consensus meeting, it is considered as high-quality evidence.

In specific recomendations, we used "should" or "strongly recommend" for strong recommendations; whereas, "suggest" or "consider" was used for weak ones.

2.9 Drafting and publishing the guideline

This guideline was published in both Chinese and English versions at the same time. Due to space limitations, the current standard revision does not include evidence descriptions. The full revision will be published in *New Medicine* (Chinese name: Yixue Xinzhi; http://www.jnewmed.com/), Volume 30 and Issue 1 2020 [7].

3 Epidemiological characteristics

3.1 Scope of the 2019-nCoV infection outbreak

Since December 2019, multiple cases occurring unexplainable pneumonia were successively reported in some hospitals in Wuhan city with a history of exposure to a large Hua'nan seafood market in Wuhan city, Hubei province, China. It has been confirmed to be an acute respiratory infection caused by a novel coronavirus. So far, the number of cases without a history of the Hua'nan seafood market exposure is increasing. In addition, clustered cases and confirmed cases without a history of travel to Wuhan emerged. Also, confirmed cases without clear exposure to the Wuhan seafood market have been found in many foreign countries or regions [8].

At 24:00 on 26 January 2020, the National Health Commission of the People's Republic of China has recorded a total of 2744 confirmed cases of pneumonia with 2019-nCoV infection from 30 provinces (districts and cities), including 461 severe cases and 80 deaths. A total of 51 cases have been cured and discharged. At present, 5794 suspected cases were recorded, 32,799 with close contacts to the confirmed patients have been tracked, 583 people were released from medical observation that day, and 30,453 people were still undergoing medical observation. A total of confirmed cases were reported from Hong Kong, Macao and Taiwan of China: 8 cases in Hong Kong, 5 cases in Macao, and 4 cases in Taiwan. In addition, confirmed cases had been reported from abroad: 7 in Thailand, 4 in Australia, 4 in Singapore, 3 in France, 3 in Japan, 3 in Korea, 3 in

Table 2 Rules for grading the recommendations

Strength of recommendation and quality of evidence	Benefit *vs.* risk and burdens	Methodological quality of supporting evidence[a]	Implications
Strong recommendation, high-quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	RCTs without important limitations or overwhelming evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Strong recommendation, moderate quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	RCTs with important limitations (inconsistent results, methodological flaws, indirect or imprecise) or exceptionally strong evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Strong recommendation, low or very low quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	Observational studies or case series	Strong recommendation but may change when higher quality evidence becomes available
Weak recommendation, high-quality evidence	Benefits closely balanced with risks and burden	RCTs without important limitations or overwhelming evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients' or societal values
Weak recommendation, moderate quality evidence	Benefits closely balanced with risks and burden	RCTs with important limitations (inconsistent results, methodological flaws, indirect or imprecise) or exceptionally strong evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients' or societal values
Weak recommendation, low or very low quality evidence	Uncertainty in the estimates of benefits, risks and burden; benefits, risk and burden may be in a closely balanced	Observational studies or case series	Very weak recommendations; other alternatives may be equally reasonable

RCTs randomized controlled trials
[a]The evidence agreed on by more than 70% frontline clinicians in consensus meeting is viewed as high-quality evidence

Malaysia, 3 in the United States, 2 in Vietnam, and one in Nepal [9].

3.2 Host and reservoir

Wild animal, bats [10] is the most possible host of the 2019-nCoV. It requires further confirmation whether pneumonia infected by the 2019-nCoV is transmitted directly from bats or through an intermediate host. It is believed that clarifying the source of the virus will help determine zoonotic transmission patterns [11].

3.3 Route of transmission

Up to present, the main infection source was the patients who with pneumonia infected by the 2019-nCoV. Respiratory droplet transmission is the main route of transmission, and it can also be transmitted through contact [12]. Although many details, such as the source of the virus and its ability to spread between people remain unknown, an increasing number of cases show the signs of human-to-human transmission [8, 13].

3.4 Etiology and pathogenesis

The 2019-nCoV isolated from the lower respiratory tract of patients with unexplainable pneumonia in Wuhan, and it is a novel coronavirus belonging to the β genus. The 2019-nCoV has an envelope; its particles are round or oval, often polymorphic, with a diameter from 60 nm to 140 nm. Its genetic characteristics are significantly different from SARSr-CoV (SARS related coronaviruses) and MERSr-CoV (MERS related coronaviruses). Current research shows it has more than 85% homology with SARSr-CoV (bat-SL-CoVZC45). 2019-nCoV can be found in human respiratory epithelial cells 96 h after in vitro isolation and culture, while it takes about 6 days in VeroE6 or Huh-7 cell lines [12].

The source of the virus, the time span of the patients discharging infective virus, and the pathogenesis are still not clear [14].

3.5 Molecular epidemiology

No evidence of viral mutation has been found so far [14]. It is necessary to obtain much more clinically isolated viruses with time and geographical variety to assess the extent of the virus mutations, and also whether these mutations indicate adaptability to human hosts [11].

3.6 Incubation and contagious period

Based on currently epidemiological survey, the latency period is generally from 3 to 7 days, with a maximum of 14 days [10]. Unlike SARSr-CoV, 2019-nCoV is contagious during the latency period [15].

3.7 Prognostic factors

The population is generally susceptible to the virus. The elderly and those with underlying diseases show more serious conditions after infection, and children and infants also get infected by the 2019-nCoV. From current knowledge of the cases, most patients have a good prognosis, the symptoms of children are relatively mild, and a few patients are in critical condition. Death cases are more frequently seen in the elderly and those with chronic underlying diseases [12].

The newest study including the first 41 confirmed cases admitted to Wuhan between 16 December 2019 and 2 January 2020 showed the median age of patients was 49 years; and the main underlying diseases were diabetes, hypertension, and cardiovascular diseases. Of them, 12 cases experienced acute respiratory distress syndrome (ARDS), 13 cases were admitted to the intensive care unit (ICU), and 6 cases died [16].

4 Screening for diseased cases and preventive measures for population

4.1 Case definitions

4.1.1 Suspected case

Patients with any 2 of the following clinical features and any epidemiological risk: (1) clinical features: fever, imaging features of pneumonia, normal or reduced white blood cell count, or reduced lymphocyte count in the early stages of the disease onset. (2) epidemiologic risk: a history of travel to or residence in Wuhan city, China or other cities with continuous transmission of local cases in the last 14 days before symptom onset; contact with patients with fever or respiratory symptoms from Wuhan city, China or other cities with continuous transmission of local cases in the last 14 days before symptom onset; or epidemiologically connected to 2019-nCoV infections or clustered onsets [12].

4.1.2 Confirmed case

Those with one of the following pathogenic evidence is the confirmed case: (1) positive for the 2019-nCoV by the real-time PCR test for nucleic acid in respiratory or blood samples [17]. 2) viral gene sequencing shows highly homogeneity to the known 2019-nCoV in respiratory or blood samples [12].

4.1.3 Clustered cases

Suspected clustering cases are defined when one confirmed case and at the same time, one or more cases of fever or respiratory infection are found in a small area (such as a family, a construction site, a unit, etc.) within 14 days.

Under the above circumstances, 2 or more confirmed cases are found, and there is the possibility of human-to-human transmission due to close contact or infection due to co-exposure, then it is determined as a clustered case [8, 18].

4.1.4 Close contacts

Those who have one of the following contacts after the onset of confirmed cases in the absence of effective protection [18]: (1) those who live, study, work, or have close contact with the confirmed cases, or other close contacts such as working closely with or sharing the same classroom or living in the same house with the confirmed case. (2) medical and nursing staffs and their family members living with them, who treated, nursed or visited the confirmed case, or other personnel who have similar close contact with the case, such as providing direct treatment or care of the case, visiting the case or staying in a closed environment where the cases are located; other patients or caregivers in the same room with the case. (3) people who have close contact with the patients in a same transport vehicle, including those who had taken care of the patients on the vehicle; the person who had companied the patients (family members, colleagues, friends, etc.); other passengers and traffic staff considered having likely close contact with the patients by investigation and evaluation. (4) other circumstances considered to be closely contacted with the person with close contact with the patients by the professional investigation and evaluation.

4.1.5 Suspicious exposure

Persons with suspicious exposure are those who are exposed without effective protection to processing, sales, handling, distributing, or administrative management of wild animals, materials, and the environments that are positive for the 2019-nCoV test [18].

4.2 Prevention

4.2.1 Persons with close contacts and suspicious exposure

Persons with close contacts and suspicious exposure should be advised to have a 14-day health observation period, which starts from the last day of contact with the 2019-nCoV infected patients or suspicious environmental exposure. Once they display any symptoms, especially fever, respiratory symptoms such as coughing, shortness of breath, or diarrhea, they should reach out for medical attention immediately [19]. Contact surveillance should be carried out for those who had exposed to accidental contact, low-level exposure to suspected or confirmed patients, i.e. checking any potential symptoms when carrying out daily activities [20]. See Table 3 for details [21].

4.2.2 Patients with suspected 2019-nCoV infection

Patients with a suspected infection should be isolated, monitored, and diagnosed in hospital as soon as possible. Doctors should make recommendations based on the patient's situation. Patients with mild symptoms and suspected infection may consider in-home isolation and home care (*weak recommendation*). Suspected infected with severe symptoms and those who need to stay in

Table 3 Recommendations for those with close contacts and suspicious exposures

No.	Recommendation items	Recommendation strength
1	Strictly take the observation period of 14 days, and go to the hospital for diagnosis and treatment if symptoms appear (fever, cough, etc.).	Strong
2	If available, inform the designated hospital in advance to send cars to pick up the patients with symptoms to the hospital.	Weak
3	Patients should wear N95 masks (priority strategy).	Strong
4	Using disposable surgical mask (alternative strategy).	Weak
5	Avoid taking public transportation to the hospital, choose an ambulance or private vehicle, and open vehicle windows for ventilation on the way to the hospital (priority strategy).	Strong
6	When walking on the road or waiting in the hospital, try to stay away from other people (at least 1 m away) and wear a mask.	Strong
7	The family members accompanying those for inspection should immediately follow the monitoring recommendations to close contacts, keep the respiratory hygiene and clean their hands properly.	Strong
8	The community or street hospital should be informed before the suspected contacts to the hospital. The vehicle used should be cleaned and disinfected with 500 mg/L chlorine-containing disinfectant, and the window should be opened for ventilation.	Strong

hospital for observation by doctor's judgment should follow the isolation guidelines for suspected patients (see Tables 4 and 5 for details).

It should also be noted that: (1) whether the suspected patients should be given in-home isolation and care or not requires careful clinical evaluation and safety assessment by professionals. (2) if the suspected patients do not get improvement in the symptoms or even worsened in condition during home care, they need to go to the doctor for treatment. (3) during the period of home care, the patients' medication and symptoms should be closely recorded and their caregivers should also monitor their body temperature daily.

Table 4 Criteria to define patients with suspected mild symptoms

No.	Definition of suspected patients with mild symptoms
1	In-home isolation and care after assessment by doctor (golden standard)
2	With a fever < 38 ℃
3	The fever can go down by itself
4	No dyspnea, no asthma
5	With or without cough
6	No underlying chronic diseases, *e.g.*: heart, lung and kidney diseases

Throughout the period of home care, healthcare personnel should perform regular (*e.g.*, daily) follow-up through face-to-face visits or phone interviews (ideally, if feasible) to follow the progress of symptoms and, if necessary, specific diagnostic tests should be conducted [14, 19, 21].

4.2.3 Prevention for travellers (Strong recommendation)

International visitors should take routine precautions when entering and leaving the affected areas, including avoiding close contacts with people with acute respiratory infection, washing hands frequently, especially after contacting with the sick or their surrounding environment; following appropriate coughing etiquette; and avoiding close contact with live or dead farming animals or bats or other wild animals [22, 23]. Passengers should avoid unnecessary travel as possible.

If they had travelled to Hubei Province (especially Wuhan city) in the past 14 days and is in fever, cough or difficulty in breathing, they should: (1) see a doctor immediately; (2) call the doctor about his/her recent trips and symptoms before going to the doctor's office or emergency room; (3) avoid contact with others; (4) not to travel around; (5) cover mouth and nose with a tissue or sleeve (not hands) when coughing or sneezing; and (6) wash hands with soap and water for at least 20 s. If soap and water are not available, use alcohol-based hand sanitizers [24].

5 Diagnosis of the 2019-nCoV cases

5.1 Clinical manifestation

The 2019-nCoV infected cases have symptoms like fever, fatigue, dry cough, dyspnea etc., with or without nasal congestion, runny nose or other upper respiratory symptoms [13, 16]. Despite the atypical symptoms were reported [25], Nan-Shan Zhong, the academician of Chinese Academy of Engineering in an exclusive interview with Xinhua News Agency on 28 January 2020, pointed out that fever is still the typical symptom of 2019-nCoV infection.

5.2 Physical examination

Patients with mild symptoms may not present positive signs. Patients in severe condition may have shortness of breath, moist rales in lungs, weakened breath sounds, dullness in percussion, and increased or decreased tactile speech tremor, etc.

5.3 Imaging examination

5.3.1 CT imaging (strong recommendation)

The imaging findings vary with the patient's age, immunity status, disease stage at the time of scanning, underlying diseases, and drug interventions.

Table 5 Home care and isolation guidelines for suspected patients with mild symptoms

No.	Recommendation items	Recommendation strength
Suspected patients with mild symptoms		
1	Well-ventilated single rooms (preferred strategy).	Strong
2	Maintain a bed distance of at least 1 m from the patient (alternative strategy).	Weak
3	Clean and disinfect household articles using 500 mg/L chlorine-containing disinfectant frequently every day (wide range).	Strong
4	Limit visits by relatives and friends.	Strong
5	The caregiver should be a healthy family member without underlying diseases.	Weak
6	Restrict the patient's activity	Strong
7	Open windows for ventilation in shared areas such as toilets and kitchens.	Strong
8	Avoid sharing toothbrush, towel, tableware, bed sheet and other items with patients. The patient's daily necessities are for single use only and should be placed separately from that of their family members.	Strong
9	When coughing or sneezing, it is necessary to wear a medical mask, or cover with a paper towel and bent elbow, and clean hands immediately after coughing and sneezing.	Strong
10	N95 masks should be worn in the same room with patients (preferred strategy).	Strong
11	Disposable surgical mask (alternative strategy). Use the mask in strict accordance with the instruction manual.	Weak
12	After washing hands with running water, dry them with a paper towel (preferred strategy).	Strong
13	Dry with a towel, and wash and disinfect the towel daily (alternative strategy).	Weak
Home caregivers		
1	Clean and disinfect hands after contact with the patient, before leaving patient's room or the house, before and after eating, after using the toilet and after entering house from outside (for visible contaminant on hands, wash hands with running water then use hand disinfection).	Strong
2	Avoid direct contact with patient's secretions or discharges, especially oral or respiratory discharges; avoid direct contact with patient's feces.	Strong
3	Wear disposable gloves (double layers) when providing oral and respiratory care to patients, handling patient's feces and urine, and cleaning the patient's room, etc. Wash hands before wearing gloves and after removing the gloves.	Strong
4	Wash the patient's clothes, bed sheets, bath towels, towels, etc. with ordinary washing soap and water, or use a washing machine at 60–90 ℃ with ordinary household washing liquid (Strong recommendation), or routinely wash them with washing machine after soaking in low concentration disinfectant (Weak recommendation).	Strong/Weak
5	Put the contaminated bedding into the laundry bag. Do not shake contaminated clothing and avoid direct contact.	Strong
6	The waste generated by the patient should be put into the closed garbage bags and replaced frequently.	Strong

The imaging features of lesions show: (1) dominant distribution (mainly subpleural, along the bronchial vascular bundles); (2) quantity (often more than three or more lesions, occasional single or double lesions); (3) shape (patchy, large block, nodular, lumpy, honeycomb-like or grid-like, cord-like, etc.); (4) density (mostly uneven, a paving stones-like change mixed with ground glass density and interlobular septal thickening, consolidation and thickened bronchial wall, etc.); and (5) concomitant signs vary (air-bronchogram, rare pleural effusion and mediastinal lymph nodes enlargement, etc.).

5.3.2 Clinical data from Zhongnan Hospital of Wuhan University

Typical CT/X-ray imaging manifestation, including

(1) Multiple, patchy, sub-segmental or segmental ground-glass density shadows in both lungs. They were classified as "paving stone-like" changes by fine-grid or small honeycomb-like thickening of interlobular septa. The thinner the CT scan layers, the clearer the ground-glass opacity and thickening of interlobular septa were displayed. A slightly high-density and ground-glass change with fuzzy edge in the fine-grid or small honeycomb-like thickening of interlobular septa were presented by the high-resolution computed tomography (HRCT), (Fig. 1: 45 cases, 54.2% in a total of 83 cases). The resolution of X-ray was worse lower than that of CT in the resolution, which was basically manifested as ground-glass

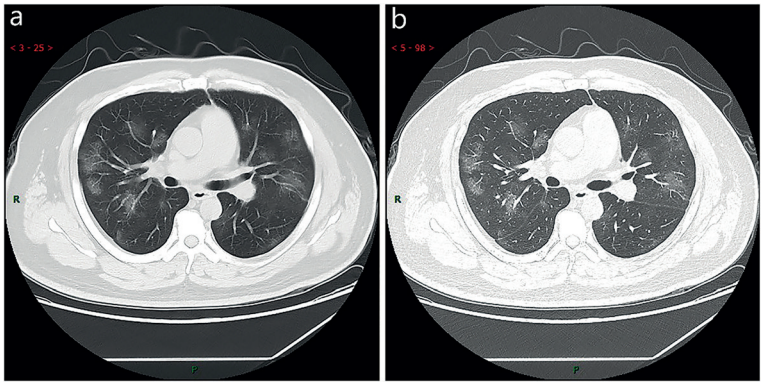


Fig. 1 Typical CT imaging manifestation (case 1). A 38 years old male with fever without obvious inducement (39.3 ℃), dry cough and shortness of breath for 3 days. Laboratory test: normal white blood cells (6.35×10^9/L), decreased lymphocytes percentage (4.1%), decreased lymphocytes count (0.31×10^9/L), decreased eosinophil count (0×10^9/L)), increased C-reaction protein (170.91 mg/L), increased procalcitonin (0.45 ng/ml). Imaging examination: multiple patches, grid-like lobule and thickening of interlobular septa, typical "paving stone-like" signs. **a** SL(Slice): 6 mm; **b** high-resolution computed tomography(HRCT). HRCT. high-resolution computed tomography

opacities with fuzzy edge (Fig. 2: 9 cases, 10.8% in a total of 83 cases).

(2) Multiple, patchy or large patches of consolidation in both lungs, with a little grid-like or honeycomb-shaped interlobular septal thickening, especially in the middle and lower lobes (Fig. 3: 26 cases, 31.3% in a total of 83 cases). It was more common in the elderly or severe condition patients.

Atypical CT/X-ray imaging manifestation, including

(1) Single, or multiple, or extensive subpleural grid-like or honeycomb-like thickening of interlobular septum, thickening of the bronchial wall, and tortuous and thick strand-like opacity. Several patchy consolidations, occasionally with a small amount pleural effusion or enlargement of mediastinal lymph nodes, can be seen (Fig. 4: 6 cases, 7.2% in a total of 83 cases). This is mostly seen in the elderly.

(2) Single or multiple solid nodules or consolidated nodules in the center of lobule, surrounded by ground-glass opacities (Fig. 5: 5 cases, 6.2% in a total of 83 cases).

Stage based on CT image The CT imaging demonstrates 5 stages according to the time of onset and the response of body to the virus, including:

(1) Ultra-early stage. This stage usually refers to the stage of patients without clinical manifestation, negative laboratory test but positive throat swab for 2019-nCoV

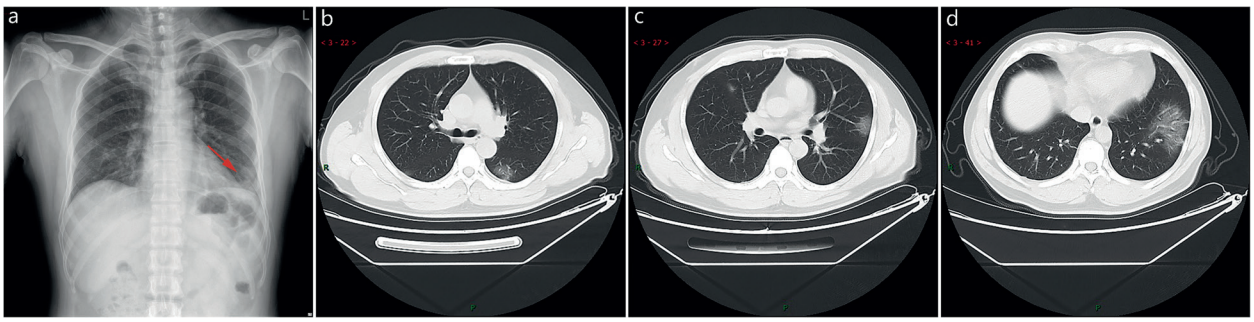


Fig. 2 Typical CT / X-ray imaging manifestation (case 2). A 51 years old male with general muscle ache and fatigue for 1 week, fever for 1 day (39.1 ℃), anemia. Laboratory test: normal white blood cells (9.24×10^9/L), lymphocytes percentage (5.1%), decreased lymphocytes (0.47×10^9/ L), decreased eosinophil count (0×10^9/L), increased C-reaction protein (170.91 mg/L), increased procalcitonin (0.45 ng/ml), increased erythrocyte sedimentation rate (48 mm/h). Imaging examination: **a** shows patchy shadows in the outer region of the left lower lobe, **b** shows large ground-glass opacity in the left lower lobe, and **c** shows subpleural patchy ground-glass opacity in posterior part of right upper lobe and lower tongue of left upper lobe, **d** shows large ground-glass opacity in the basal segment of the left lower lobe

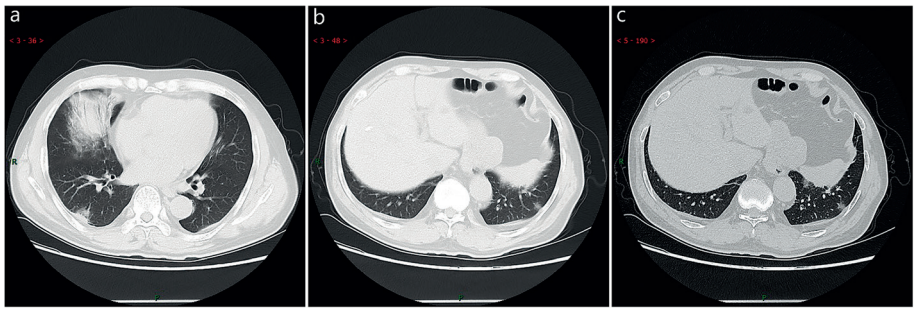


Fig. 3 Typical CT / X-ray imaging manifestation (case 3). A 65 years old male with fever for 4 days (38.7 ℃). Laboratory test: normal white blood cells (3.72×10^9/L), decreased lymphocytes (0.9×10^9/ L), decreased eosinophil count (0×10^9/L), increased C-reaction protein (53.0 mg/L), increased procalcitonin (0.10 ng/ml), reduced liver function, hypoproteinemia, and mild anemia. Imaging examination: **a** and **b** showed large consolidation in the right middle lobe, patchy consolidation in the posterior and basal segment of the right lower lobe, with air-bronchogram inside, **c** showed patchy consolidation in the outer and basal segment of the left lower lobe, and a small amount of effusion in the right chest

within 1–2 weeks after being exposed to a virus-contaminated environment (history of contact with a patient or patient-related family members, unit, or medical staff in a cluster environment). The main imaging manifestations are single, double or scattered focal ground-glass opacity, nodules located in central lobule surrounded by patchy ground-glass opacities, patchy consolidation and sign of intra-bronchial air-bronchogram, which was dominant in the middle and lower pleura (Fig. 6: 7 cases, 8.4% in a total of 83 cases).

(2) Early stage.This stage refers to the period of 1–3 days after clinical manifestations (fever, cough, dry cough, etc.). The pathological process during this stage is dilatation and congestion of alveolar septal capillary, exudation of fluid in alveolar cavity and interlobular interstitial edema. It showed that single or multiple scattered patchy or agglomerated ground-glass opacities, separated by honeycomb-like or grid-like thickened of interlobular septa (Fig. 7: 45 cases, 54.2% in a total of 83 cases).

(3) Rapid progression stage. This stage refers to the period about 3–7 days after clinical manifestations started, the pathological features in this stage are the accumulation of a large number of cell-rich exudates in the alveolar cavity, vascular expansion and exudation in the interstitium, both of which lead to further aggravation of alveolar and Interstitial edema. The fibrous exudation connects each alveolus through the inter-alveolar space to form a fusion state. The CT manifested a fused and large-scale light consolidation with air-bronchogram inside (Fig. 8: 17 cases, 20.5% in a total of 83 cases).

(4) Consolidation stage. This stage refers to the period around 7–14 days after clinical manifestations appeared. The main pathological features in this stage are the fibrous exudation of the alveolar cavity and the disappearance of capillary congestion in the alveolar wall. CT imaging showed the multiple patchy consolidations in slighter density and smaller range than that of the previous stage. (Fig. 9: 26 cases, 31.2% in a total of 83 cases).

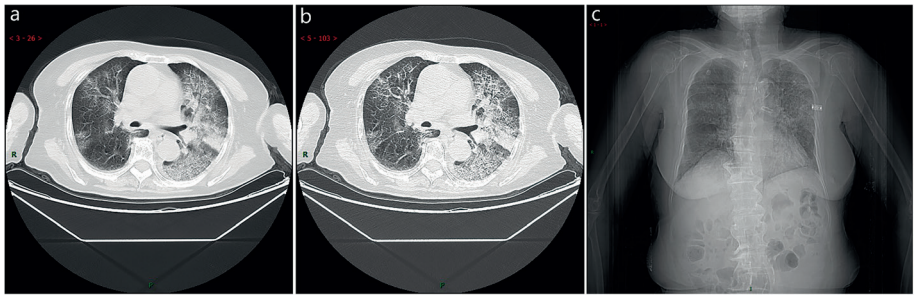


Fig. 4 Atypical CT / X-ray imaging manifestation (case 1). An 83 years old female with fever for 4 days (maximum temperature of 38.8 ℃), cough, chills, sore throat, dry cough for 1 week, chest tightness and shortness of breath aggravating for 1 week. Laboratory test: normal white blood cells (4.6×10^9/L), normal neutrophil percentage (65.8%), decreased lymphocytes percentage (19.9%). Imaging examination: **a** and **b** showed diffuse interlobular septum thickening in both lungs to form a grid opacity, thickening of bronchial wall, and consolidation in the left sublobal lung. **c** showed diffused grid-like opacities in both lungs, especially in the left lung

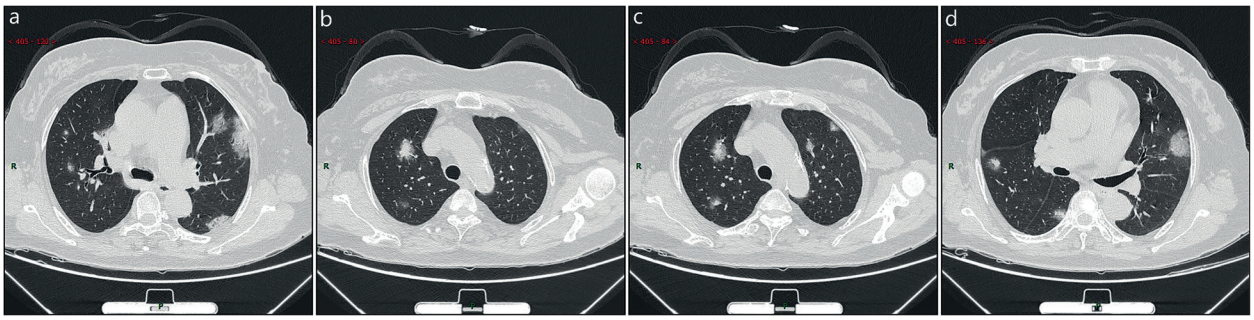


Fig. 5 Atypical CT / X-ray imaging manifestation (case 2). A 56 years old female with fever for 3 days. Laboratory test: decreased total protein (54.0 g/L),decreased albumin (35.5 g/L),decreased globulin (18.5 g/L), normal white blood cells (4.87 × 10^9/L), decreased lymphocytes percentage (10.1%), decreased lymphocytes (0.49 × 10^9/ L), decreased eosinophil count (0 × 10^9/L)), decreased eosinophil count percentage (0%). Imaging examination: **a** showed two consolidation nodulesat the center of the lateral segment of middle lobe of the right lung which was surrounded by ground-glass opacities; **b** showed patchy ground-glass opacity in the anterior segment of the right upper lung with patchy consolidation lesions in it; **c** showed patchy ground-glass opacities in both lungs with patchy consolidation lesions in it. **d** showed patchy consolidation in the ground-glass opacities in the middle lobe and dorsal segment of lower lobe of right lung

(5) Dissipation stage. This stage refers to the period roughly between 2 and 3 weeks after the onset of clinical manifestations. The range of lesions was further reduced. CT imaging showed patchy consolidation or strip-like opacity. As time goes on, it showed grid-like thickening of interlobular septum, thickening and strip-like twist of bronchial wall and a few scattered patchy consolidations (Fig. 10: 17 cases, 20.5% in a total of 83 cases).

5.4 Differential diagnosis

It mainly should be distinguished from other known viral virus of pneumonia, such as influenza viruses, parainfluenza virus, adenovirus, respiratory syncytial virus, rhinovirus, human metapneumovirus, SARSr-CoV, etc.; and also from mycoplasma pneumonia, chlamydia pneumonia, and bacterial pneumonia. In addition, it should be distinguished from non-infectious diseases, such as vasculitis, dermatomyositis, and organizing pneumonia.

5.5 Techniques for laboratory tests

5.5.1 Hematology examination

In the early stage of the disease, the total number of leukocytes decreased or keeps normal, with decreased lymphocyte count or increased or normal monocytes. High attention should be paid on the situation where the absolute value of lymphocyte is less than 0.8 × 10^9/L, or the numbers of CD4 and CD8 T cells are significantly decreased, which generally recommend rechecking the blood routine changes after 3 days.

5.5.2 Detection of pathogens in respiratory tract

(1) Flu antigens. At present, routinely detected flu antigens are A, B, and H7N-subtypes. Sampling of throat swabs is conducive to early rapid screening for flu because of the fast test, but it has a relatively high false negative rate.
(2) Respiratory virus nucleic acid. The detection of respiratory virus nucleic acid is commonly used to

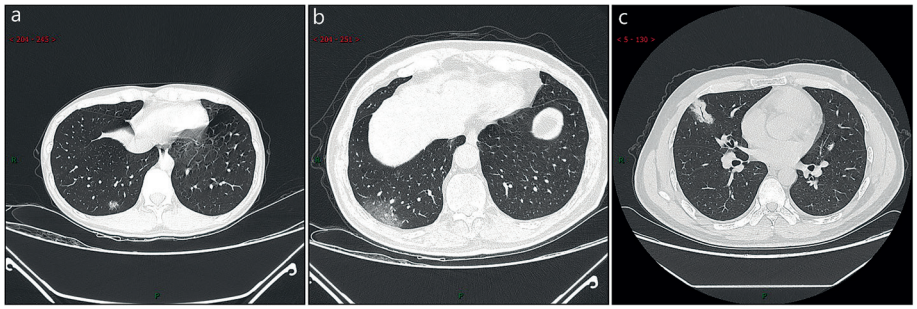


Fig. 6 CT imaging of ultra-early stage. **a** A 33 years old female with patchy ground-glass opacities after occupational exposure. **b** A 67 years old male with a history of contact with infected patients, showing large ground-glass opacity. **c** A 35 years old female exhibiting large consolidated opacity with air-bronchogram inside after occupational exposure

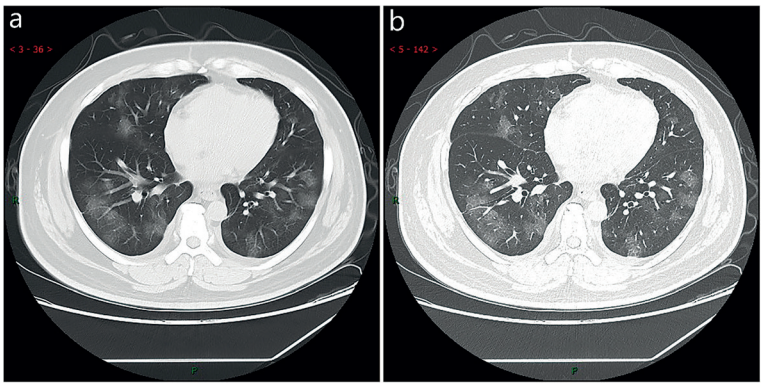


Fig. 7 CT imaging of early stage. Male, 38 years old, fever without obvious inducement (39.3 ℃), dry cough and shortness of breath for 3 days. Laboratory test: decreased white blood cells (3.01×10^9/L), decreased lymphocytes (0.81×10^9/ L), increased C-reaction protein (60.8 mg/L), increased procalcitonin (0.16 ng/ml). Imaging examination: **a** (thin layer CT) and **b** (high-resolution CT) showed multiple patchy and light consolidation in both lungs and grid-like thickness of interlobular septa

detect the infection by other common respiratory viruses, mycoplasma and chlamydia infection, such as adenovirus, parainfluenza virus, respiratory syncytial virus, mycoplasma, chlamydia, influenza A and influenza B virus, etc.

(3) 2019-nCoV nucleic acid detection. Accurate RNA detection of 2019-nCoV is with diagnostic value (*Strong recommendation*). The RNA of 2019-nCoV positive in the throat swab sampling or other respiratory tract sampling by fluorescence quantitative PCR method, especially that from multiple samples and detection kits, excluding sample quality, sample collection time, contaminatory and technical problems, is of great support for etiological diagnosis.

(4) Other laboratory testing. There are other laboratory tests for the status of 2019-nCoV infection, including blood gas analysis, liver and kidney function, myocardial enzyme, myoglobin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Procalcitonin (PCT), lactate, D-dimer, coagulation image, urine routine test, inflammatory factors (interleukin(IL)-6, IL-10, TNF - α), 11 items of tuberculosis (TB) subgroup, complement, anti-acid staining, etc. Blood gas analysis is helpful to judge the oxygenation of moderately-illed and severe patients. Combining the increase of lactic acid, it is feasible to screen the patients with high-risk of oxygenation disorder. Some infected patients have increased liver enzymes, muscle enzyme, ESR and myoglobin. The detection of CRP and PCT is of certain value to distinguish whether there was bacterial infection in the lung. D-dimer of most severe patients was significantly increased in this epidemic, with frequent clotting disorders and microthrombotic formation in peripheral blood

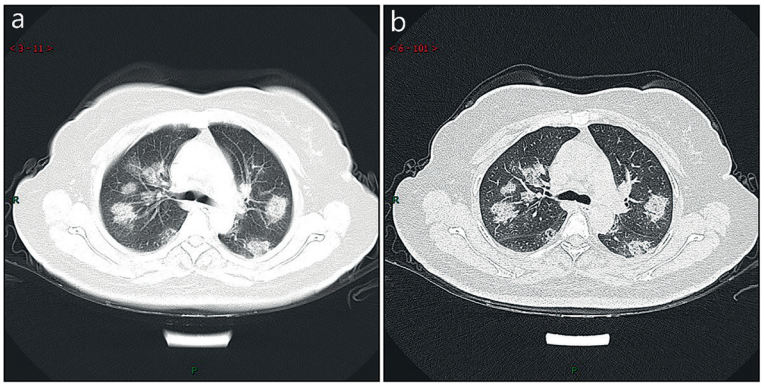


Fig. 8 CT imaging of rapid progression stage. A 50 years old female with anorexia, fatigue, muscle soreness, nasal congestion and runny nose for 1 week, sore and itching throat for 2 days. Laboratory test: increased erythrocyte sedimentation rate (25 mm/h), normal white blood cells (4.08×10^9/L), decreased lymphocytes (0.96×10^9/ L), increased C-reaction protein (60.8 mg/L). Imaging examination: **a** (thin layer CT) and **b** (high-resolution CT) showed multiplepatchy and light consolidation in both lungs and grid-like thickness of interlobular septa

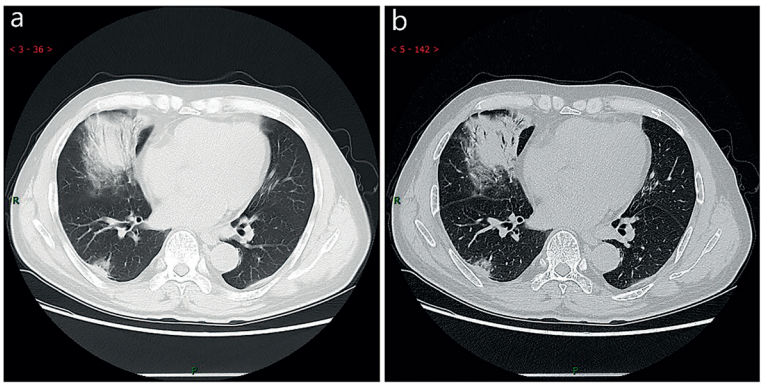


Fig. 9 CT imaging of consolidation stage. A 65 years old male with fever (maximum temperature of 39 ℃). Laboratory test: hypoproteinemia (decreased total protein (62.20 g/L), decreased albumin (35.70 g/L)), abnormal liver function (increased alanine aminotransferase (79 U/L), increased aspartate aminotransferase (72 U/L)), increased procalcitonin (0.10 ng/ml), increased C-reaction protein (53 mg/L), decreased white blood cells (3.72×10^9/L), decreased lymphocytes (0.9×10^9/ L), mildanemia (decreased red blood cells (4.10×10^{12}/L), decreased hemoglobin (131.10 g/L), decreased hematocrit (39.0%). Imaging examination: **a** (thin layer CT) and **b** (high-resolution CT) showedmultiple patchyand large consolidation in right middle lobe, posterior and basal segment of right lower lobe and outer and basal segment of left lower lobe, with air-bronchogram inside

vessels. Detection of other inflammatory factors may help to preliminarily evaluate the immune status of patients.

5.5.3 Clinical data from Zhongnan Hospital of Wuhan University

In the early stage of this disease, the total number of leukocytes in peripheral blood was normal or decreased, and the lymphocyte count decreased. In some patients, liver enzyme (transaminase), creatine kinase (CK) and myoglobin increased. CRP, ESR, and IL-6 increased, and PCT was normal in most patients. The increased D-dimer occurred in severe cases.

The data from the first 38 patients with 2019-nCoV infection who hospitalized in Zhongnan Hospital of Wuhan University were collected. Analysis revealed that the average value of white blood cells (WBC) was 5.45 (2.30–13.82) $\times 10^9$/L, PLT was 164.5 (47–317) $\times 10^9$/L, lymphocyte was 0.87 (0.24–2.27) $\times 10^9$/L, and monocyte was 0.38 (0.12–0.62) $\times 10^9$/L. The average value of ALT (alanine aminotransferase) was 37.6 (6–128) U/L and AST (aspartate aminotransferase) was 53.3 (18–169) U/L. The average value of CK was 315 (33–3051) U/L, ESR was 29.3 (8–67) mm/h, CRP was 61.8 (3–170.91) mg/L, IL-6 was 57 (3.1–134.4) pg/ml, and D-dimer was 400 (46–3330) ng/ml.

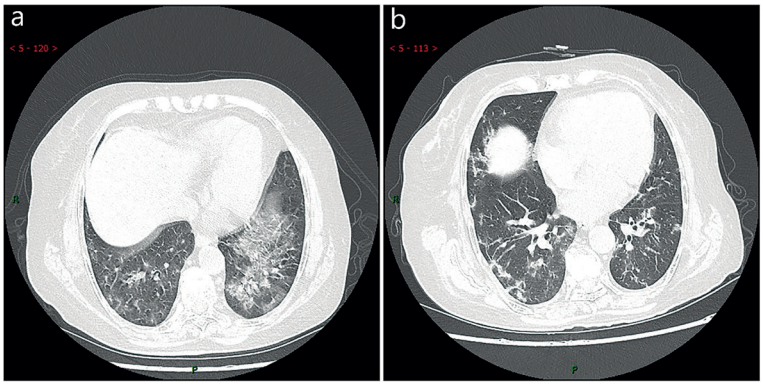


Fig. 10 CT imaging of dissipation stage. A 79 years old female with intermittent fever. Laboratory test after 3 days of comprehensive treatment: decreased red blood cells (3.73×10^{12}/L), hemoglobin (107 g/L), decreased hematocrit (31.8%), decreased lymphocytes percentage (13.9%), decreased lymphocytes (0.62×10^9/ L), decreased eosinophil count percentage (0%), decreased eosinophil count (0×10^9/L), increased alanine aminotransferase (46 U/L), deceased total protein (56.8 g/L), decreased albumin (33.5 g/L), normal C-reaction protein and procalcitonin. Imaging examination: **a** patchy ground-glass opacity and grid-like thickening of interlobular septa in the tongue-like segment of left upper lobe, and patchy consolidation in the posterior segment of right middle and lower lobe. **b** 9 days after admission to hospotial, CT scan showed absorption of lesions in the middle lobe, narrowing of lesions in the lower lobe of the right lung, and absorption of lesions in the tongue-like segment of left upper lobe which exhibited a cord-like change

Compared with 120 healthy check-ups, the absolute value of lymphocyte (0.87 vs 2.13) × 10^9/L, lymphocyte percentage (19.5% *vs* 33.7%), eosinophil percentage (0.13% *vs* 2.16%), and absolute value (0.0061 *vs* 0.1417) × 10^9/L in 2019-nCoV patients were significantly reduced ($P < 0.05$). The absolute number (4.2 *vs* 3.7) × 10^9/L and the percentage (72.0% *vs* 57.0%) increased in 2019-nCoV patients ($P < 0.05$). The percentage of monocytes increased slightly (8.1% *vs* 6.8%), while the absolute number of monocytes did not change significantly (0.38 *vs* 0.44) × 10^9/L.

5.6 Other early diagnosis methods

The next generation sequencing (NGS) and electron microscope technology play a role in the early diagnosis, but their diagnostic values have been weakened by the discovery of specific nucleic acid detection technology. In addition, NGS detection can tell whether the pathogen has mutated or not.

6 Treatment and control

6.1 Principles

Suspected and confirmed cases need to be treated in designated hospitals with effective isolation and protection conditions. Suspected cases need to be treated separately in single room, confirmed cases are admitted to a same ward, and critical cases should be admitted to ICU as soon as possible.

6.2 Treatment plans

(1) The patient should rest in bed, being monitored for vital signs (heart rate, pulse oxygen saturation, respiratory rate, blood pressure) and given supportive treatment to ensure sufficient energy intake and balance for water, electrolytes, acid-base levels and other internal environment factors *(Strong recommendation).*
(2) The patient should be monitored for blood routine, CRP, PCT, organ function (liver enzyme, bilirubin, myocardial enzyme, creatinine, urea nitrogen, Urine volume, etc.), coagulation function, arterial blood gas analysis and chest imaging *(Strong recommendation).*
(3) The patient should be given effective oxygen therapy, including nasal catheter, mask oxygen, high flow nasal oxygen therapy (HFNO), non-invasive ventilation (NIV) or invasive mechanical ventilation *(Strong recommendation).*

First, oxygen therapy is the choice for patients with severe respiratory infections, respiratory distress, hypoxemia or shock. The initial flow rate is 5 L/min, and the titration flow rate is to reach the target oxygen saturation (adults: $SpO_2 \geq 90\%$ in non-pregnant patients, $SpO_2 \geq 92–95\%$ in pregnant patients; children: $SpO_2 \geq 94\%$ in children with obstructive dyspnea, apnea, severe respiratory distress, central cyanosis, shock, coma or convulsions, and ≥ 90% in other children).

Second, respiratory support should be given to patients with hypoxic respiratory failure and acute respiratory distress syndrome. HFNO or NIV can be selected when nasal cannula or mask oxygen therapy was ineffective or the patient had hypoxic respiratory failure. However, when patients had hypercapnia (acute exacerbation of chronic obstructive pulmonary disease, cardiogenic pulmonary edema), hemodynamic instability, multiple organ failure, and abnormal mental status HFNO oxygen is not the routinely adopted measure. If respiratory failure cannot be improved or worsens continuously within a short time (1 h) after using HFNO or NIV, intubation should be performed immediately. Low tidal volume (4-8 ml/kg) and low suction pressure (platform pressure < 30cmH_2O) are used for invasive mechanical ventilation. It is suggested that positive end-expiratory pressure (PEEP) with high positive end-expiratory pressure should be used in patients with moderate or severe acute respiratory distress syndrome, and PEEP should be titrated according to FiO_2 to maintain SpO_2, in order to improve alveolar atelectasis and reduce alveolar hyper-expansion and pulmonary vascular resistance at the end of inspiration. For severe patients with ARDS, it is recommended to ventilate in prone position for more than 12 h/d.

(4) Extracorporeal Membrane Oxygenation (ECMO) should be considered for the patients with refractory hypoxemia that is difficult to be corrected by protective lung ventilation. *(Strong recommendation).*

6.3 Drug treatment

6.3.1 Antiviral treatment

(1) At present, there is no evidence from RCT to support specific drug treatment against the new coronavirus in suspected or confirmed cases.
(2) The α-interferon atomization inhalation can be considered (5 million U per time for adults in sterile injection water, twice a day) *(Weak recommendation)*; lopinavir/ritonavir orally, 2 capsules each time, twice a day, can be also considered *(Weak recommendation).*

Low-level evidences included retrospective cohort, historically controlled studies, case reports, and case series revealed that lopinavir/ritonavir alone or in combination with antivirals produced certain benefits in the

treatment of SARS and MERS, such as reducing the incidence or mortality of ARDS [26–29]. A recently systematic review showed that lopinavir/ritonavir's anti-coronavirus effect was mainly seen in its early application, for reducing patient mortality and reduced glucocorticoid consumption. However, if the early treatment window is missed, there will be no significant effect in their late application [30]. Real-world study stills need to further explore the clinical effects of its early use in 2019-nCoV infected pneumonia.

The effectiveness of the combined use of antivirals is still controversial [31–34].

6.3.2 Antibiotic therapy

(1) Principles. Avoid blind or inappropriate use of antibacterial drugs, especially the combination of broad-spectrum antibacterial drugs. Enhancement of bacteriological surveillance should be performed and promptly given appropriate antibacterial drugs when it occurs secondary bacterial infection.
(2) According to the clinical manifestations of patients, if the accompanying bacterial infection cannot be ruled out, mild patients can take antibacterial drugs against community-acquired pneumonia, such as amoxicillin, azithromycin, or fluoroquinolones; empirical antibacterial treatment in severe patients should cover all possible pathogens, deescalating therapy until the pathogenic bacteria are clarified.

6.3.3 Corticosteroid therapy

The use of corticosteroids for severe ARDS is controversial; therefore, systemic use of glucocorticoids needs to be cautious. Methylprednisolone can be used as appropriate for patients with rapid disease progression or severe illness. According to the severity of the disease, 40 to 80 mg of methylprednisolone per day can be considered, and the total daily dose should not exceed 2 mg/kg *(Weak recommendation)*.

SARS management related researches showed that timely use of non-invasive continuous positive airway pressure and corticosteroids is an effective strategy for increased lung shadows and increased dyspnea. Appropriate use of glucocorticoids is able to significantly improve the clinical symptoms of patients with SARS, reduce the degree of disease progression, and accelerate the absorption of lung lesions; but it cannot shorten the length of hospital stay [35, 36]. Be cautious that hormone therapy has some incidence of adverse reactions [37].

6.3.4 Other medications

(1) Symptomatic treatment of fever. When the temperature is higher than 38.5 ℃, ibuprofen can be used for antipyretic (oral, 0.2 g per time, it can be used every 4–6 h in continuous fever, but no more than 4 times in 24 h), and the temperature below 38 ℃ is acceptable. Much lower body temperature is not conducive to antiviral treatment.
(2) Nutrition support treatment. Inpatients are screened for nutrition risk based on the NRS2002 score when they are admitted to the hospital. The recommended plan for patients with different nutrition risk scores are as follows:
First, if the total score is < 3 points, it is recommended to eat protein-rich foods (such as eggs, fish, lean meat, dairy products) and carbohydrate-containing diets. The supposed ideal energy intake is 25–30 kcal / (kg·d) and the protein mass are 1.5 g / (kg·d).
Second, if the total score is ≥3 points, the patient should be given nutritional support as early as possible. It is recommended to increase protein intake by oral nutrition supplement, 2–3 times/day (≥ 18 g protein/time). In order to reach the mount of 18 g protein/time, protein powder can be added on the basis of standard whole protein preparations. Enteral nutrition tube should to be placed when the patient cannot intake supplemental nutrition by oral routine.
(3) Reduce the incidence of stress ulcers and gastrointestinal bleeding. Use H_2 receptor antagonists or proton pump inhibitors in patients with gastrointestinal bleeding risk factors. The risk factors for gastrointestinal bleeding include mechanical ventilation ≥48 h, coagulation dysfunction, renal replacement therapy, liver disease, various complications, and a higher score of organ failure.
(4) Reduce the secretion of lung glands and improve the respiratory function. For patients with dyspnea, cough, wheeze, and respiratory distress syndrome due to the increased respiratory gland secretion, it is recommended to use selective (M1, M3) receptor anticholinergic drugs to reduce the secretion, relax the smooth muscle in airway, relieve airway spasm and improve the pulmonary ventilation.
(5) Reduce the incidence of venous embolism. Evaluate the risk of venous embolism in patients and use low-molecular-weight heparin or heparin in high-risk patients without contraindications.

6.4 Traditional Chinese medicine treatment

6.4.1 Guiding principles

Treat the patient based on syndrome differentiation individually. Prevention before illness is better than treatment after getting diseased.

6.4.2 Prevention

(1) Community. Implement relevant national regulations and take great effort to keep away from contaminated materials, disinfect the environment, and improve the healthcare management.
(2) Individual. It is recommended to take food in proper amount and balanced nutrition, have regular daily life and physical activities, and avoid overloaded work.
(3) Psychology. Develop proper interests and career in a mutual promoting manner.
(4) Drug. Including:
   i Fumigation with moxa in the room, 1-5 g/m^2 for 30 min per day.
   ii Wearing perfumed Chinese herb bags (clove, fineleaf schizonepeta herb, *Perilla frutescens*, atractylodes lancea, cinnamon, biond magnolia flower, asarum sieboldii, and *Elettaria cardamomum*, 2 g for each, crushed into powder and put it into bags for external use, change a new one every 10 days).
   iii Prescription of Chinese herbs for feet bath (vulgaris 10 g, carthamus 10 g, and dried ginger 6 g) Soaking the herbs in boiling water and bath the feet into the medical liquid when the temperature is suitable. Soak feet for about 20 min.
   iv Prescription of Chinese herbs for prophylaxis: *Astragalus mongholicus* 12 g, roasted rhizoma atractylodis macrocephalae 10 g, saposhnikovia divaricata 10 g, *Cyrtomium fortunei* 10 g, honeysuckle 10 g, dried tangerine or orange peel 6 g, eupatorium 10 g, and licorice 10 g. Taking the medicine above yielded decoction once a day for adults, and for 5 days as a treatment course. If for children, cutting the dose to half.
   v Medical tea: perilla leaf 6 g, agastache leaf 6 g, dried tangerine or orange peel 9 g, stewed amomum tsao-ko 6 g, and 3 slices of ginger. Soak the herbs in hot water and drink the water just like enjoying the tea.
   vi Chinese patent medicine: Huoxiang Zhengqi capsule or Huoxiang Zhengqi Shui (in half dose).

6.4.3 Treatment [12]

In medical observation period There are two clinical symptoms in this period, including:

(1) Clinical symptoms 1: hypodynamia accompanied by gastrointestinal upset. And the recommended Chinese patent medicine is the Huoxiang Zhengqi capsules (ball, liquid, or oral liquid).
(2) Clinical symptoms 2: hypodynamia and fever. And the recommended Chinese patent medicines is the Jinhua Qinggan granules, Lianhua Qingwen capsules (granules), Shufeng Jiedu capsules (granules), or Fangfeng Tongsheng pills (granules).

Clinical treatment period This period involving 7 stages, including:

(1) Early-stage, characterized as exterior syndrome of cold-dampness. In this stage, the clinical manifestations presents as follow: aversion to cold without sweating, headache and generalized heaviness, limb pain, glomus and fullness in the chest and diaphragm, thirst with no desire to drink, ungratifying loose stool, yellow urine, frequent micturition and yellow urine. The therapeutic logic is to dissipate cold and eliminate dampness. And the recommended prescription is the Huoxiang Zhengqi powder (Yin dampness injuring superficies case from the *National Famous Traditional Chinese Medical Doctor Medical Cases*); which comprises of perilla leaf 10 g, atractylodes lancea 15 g, radix angelicae dahuricae 10 g, dried tangerine or orange peel 10 g, notopterygium root 10 g, agastache rugosus 10 g (end addition), mangnolia officinalis 10 g, saposhnikovia divaricata 10 g, poria peel 15 g, and *Tetrapanax papyriferus* 10 g above yielded decoction. In addition, the recommended Chinese patent medicine is Huoxiang Zhengqi capsules or Huoxiang Zhengqi Shui.
(2) Early-stage, characterized as cold-dampness obstructing lung. In this stage, the clinical manifestations presents as follow: aversion to cold with or without fever, dry cough, dry throat, fatigue and hypodynamia, oppression in chest, epigastric fullness, or nausea, loose stool. The tongue is pale or reddish, the tongue fur is slimy white, and soggy pulse. Hence, the therapeutic logic is to dissipate cold and resolve obstruction. And the recommended prescriptions comprises of atractylodes lancea 15 g, dried tangerine or orange peel 10 g, mangnolia officinalis 10 g, agastache rugosus 10 g (end addition), amomum tsao-ko 6 g, ephedra herb 6 g, notopterygium root 10 g, ginger 10 g, areca-nut 10 g (end addition), periostracum cicada 10 g, bombyx batryticatus 10 g, and rhizoma curcumae longae 10 g above yielded decoction.
(3) Middle-stage, characterized as epidemic toxin blocking the lung. In this stage, its clinical manifestations includes persistent fever or alternating cold and heat, cough with less phlegm, or yellow phlegm, abdominal distension and constipation; oppression in chest with anhelation, cough with wheezes, panting on

exertion; or red tongue, slimy yellow fur or yellow dry fur, slippery and rapid pulse. Therefore, the therapeutic logic is clearing heat and detoxicating. And the recommended prescription comprises of almond 10 g, gypsum 30 g (predecoction), trichosanthes kirilowii 30 g, rhubarb 6 g (end addition), ephedra with honey fried 6 g, semen lepidii 10 g, peach kernel 10 g, amomum tsao-ko 6 g, arecanut 10 g, and atractylodes lancea 10 g above yielded decoction.

In addition, the recommended Chinese patent medicine is Xiyanping injection or Xuebijing injection.

(4) Severe stage, characterized as heat toxin generating stasis. In this stage, the clinical manifestations is known as high fever, oppression in chest with anhelation, purple-black facial complexion, lips dark and swollen, obnubilation, crimson tongue, yellow dry fur, surging and fine rapid stringlike pulse. Thus, its therapeutic logic is detoxicating and dispersing blood stasis.

The recommended prescription is three Yellows and Gypsum decoction, Shang Jiang Powder, and Toxin-Resolving Blood-quickening decoction. Its composition comprises of ephedra with honey fried 10 g, almond 10 g, gypsum 20-30 g, periostracum cicada 10 g, bombyx batryticatus 10 g, rhizoma curcumae longae 10 g, rhubarb stir-fried with wine 10 g, scutellaria baicalensis 10 g, coptis chinensis 5 g, phillyrin 15 g, angelica sinensis 10 g, peach kernel 10 g, radix paeoniae rubra 15 g, and rhizome of rehmannia 15 g above yielded decoction.

The recommended Chinese patent medicines is the Xiyanping injection, Xuebijing injection, Qingkailing injection, or Angong Niuhuang pills.

(5) Severe-stage, characterized as inner blocking causing collapse. In this stage, the clinical manifestations include dyspnea, panting on exertion or need assisted ventilation, accompanied by coma, and agitation, cold limbs with cold sweating, dark purple tongue, thick or dry thick tongue fur, floating and rootless pulse. The thrapeutic logic is rescuing from collapse by restoring Yang. Hence, the recommended prescription comprises of ginseng 15 g, aconitine 10 g (predecoction), and *Cornus officinalis* 15 g above yielded decoction, and both taken with fluid Suhexiang pills or Angong Niuhuang pills.

The recommended Chinese patent medicines is Xuebijing injection, Shenfu injection, or Shengmai injection.

(6) Recovery-stage, presents as lung and spleen Qi deficiency. Its clinical manifestations include shortness of breath, fatigue and hypodynamia, anorexia, nausea and vomiting, glomus and fullness, weak stools, ungratifying loose stool, pale tender-soft enlarged tongue, slimy white tongue fur. Therefore, therapeutic logic is to supplement the spleen and lung.

The recommended prescription comprises of rhizoma pinellinae praeparata 9 g, dried tangerine or orange peel 10 g, *Codonopsis pilosula* 15 g, radix astragali preparata 30 g, poria cocos 15 g, agastache rugosus 10 g, and fructus amomi 6 g (end addition) above yielded decoction. In addition, the recommended Chinese patent medicines is pill of costus and amomum with six noble ingredients.

(7) Recovery-stage, characterized as deficiency of Qi and Yin. The clinical manifestations of this stage is generalized heat with sweating, chest heat vexation, Qi counterflow with retching and vomiting, shortness of breath and lassitude of essence-spirit, red tongue and thin tongue fur, vacuous pulse. Hence, the therapeutic logics is boost Qi and nourish Yin.

The recommended prescription is Zhuye Shigao decoction with cogongrass rhizome and rhizoma phragmitis; and the composition of this prescription includes bamboo leaf 15 g, gypsum 15 g (predecoction), *Codonopsis pilosula* 15 g, radix ophiopogonis 10 g, pinellia ternate 9 g, cogongrass rhizome 15-30 g, rhizoma phragmitis 20 g, licorice 10 g, and polished round-grained rice 30 g above yielded decoction.

The recommended Chinese patent medicine: Shengmaiyin.

6.5 Treatment of severe patients

6.5.1 Hypoxemic respiratory failure and ARDS treatments

Treatment principle: treat the patients to improve the symptoms and underlying diseases, actively prevent potential complications and secondary infection; provide timely measures to support organ function.

(1) Hypoxic respiratory failure and severe ARDS. Give oxygen therapy immediately to patients with ARDS, and closely monitor them for signs of clinical deterioration, such as rapidly progressive respiratory failure. Consider severe hypoxemic respiratory failure when standard oxygen therapy fails. When patients have increased frequency of breathing (> 30 times/min) and hypoxemia (SpO_2 < 90% or PaO_2 < 60 mmHg) even with oxygen delivered via a face mask and reservoir bag (gas flow of 10~15 L/min, FiO_2 0.60–0.95), it may be considered as hypoxic respiratory failure.

ARDS is a status of severe acute hypoxic respiratory failure caused by increased pulmonary capillary permeability and alveolar epithelial cell damage. It can be divided into mild, moderate and severe

Table 6 The Berlin definition for acute respiratory distress syndrome

Item	Mild	Moderate	Severe
Onset time	Respiratory symptoms developed/aggravated within 1 week after clinically known damage		
Hypoxemia	PaO_2/FiO_2 201–300 mmHg, PEEP or CPAP ≥5 cmH_2O	PaO_2/FiO_2 101–200 mmHg, PEEP≥5 cmH_2O	PaO_2/FiO_2 ≤ 100 mmHg, PEEP≥10 cmH_2O
Causes of pulmonary edema	Respiratory failure cannot be completely explained by heart failure or fluid overload. Objective assessment (such as echocardiography) is needed to eliminate the possibility of hydrostatic pulmonary edema if other risk factor is absent.		
Abnormality in imaging	Decreased transparence of two lungs cannot be completely explained by pleural effusion, atelectasis or nodules.		

PEEP positive end-expiratory pressure, *CPAP* continuous positive airway pressure

conditions according to the Berlin definition [38] (Table 6).

(2) HFNO. Under the support of standard oxygen therapy, to maintain SpO_2 above 93% stills hard, and the breathing rate increases rapidly, then HFNO should be considered. HFNO can deliver 60 L/min of gas flow and FiO_2 up to 1.0. Generally, gas flow is initially set as 30–40 L/min and oxygen concentration 50%–60%, which is well tolerated and coordinated. Then settings can be adjusted according to the oxygenation status of patients. Compared with standard oxygen therapy, HFNO is able to reduce the chance of tracheal intubation. Patients with hypercapnia (like exacerbation of obstructive lung disease, cardiogenic pulmonary edema), hemodynamic instability, multi-organ failure, or abnormal mental status should not be given HFNO. HFNO may be safe in patients with mild-moderate and non-worsening hypercapnia. However, if the respiratory distress still exists or even worsens dramatically under HFNO (FiO_2 > 70%, gas flow > 50 L/min for 1 hour), the respiratory supporting strategy should be changed.

(3) NIV. NIV provides a certain positive pressure ventilation effect through the positive pressure formed by the closed mask. HFNO combined with intermittent short-term NIV (1–2 h) support may be useful to reduce respiratory power consumption and improve oxygenation. But NIV guidelines recommend the use of respiratory support therapy in hypoxemic respiratory failure or pandemic viral illness. Limited data showed a high failure rate of NIV in MERS patients. Invasive mechanical ventilation should be considered in case the ARDS still exists and even acutely deteriorates in NIV process (about 1 h). Patients with hemodynamic instability, multiple organ failure, or abnormal mental status should not receive NIV.

(4) Invasive mechanical ventilation. Under the support of HFNO (the demand for FiO_2 > 70% and gas flow > 50 L/min) or NIV, ARDS still exists and even acutely deteriorates, invasive mechanical ventilation should be implemented as soon as possible.

Endotracheal intubation should be carried out by trained and experienced provider using airborne precautions, since endotracheal intubation is an operation that may produce a large number of contagious aerosols.

The strategy of protective lung ventilation should be implemented in invasive mechanical ventilation: lower tidal volume (4–6 ml/kg), lower plateau pressure (< 30 cmH_2O), and appropriate PEEP. For patients with moderate-severe ARDS (PaO_2/FiO_2 < 150), it is recommended to use higher PEEP, apply prone ventilation for more than 12 h per day and adopt deep sedation and analgesia muscle relaxation strategy within the first 48 h of mechanical ventilation. For patients with severe acute hypoxic respiratory failure, we should pay attention to and prevent ventilator-associated lung injury after mechanical ventilation.

(5) Extracorporeal Life Support (ECLS). In the process of invasive mechanical ventilation when the patient is still in the state of hypoxia, combined with increased partial pressure of carbon dioxide (excluding ventilation dysfunction, $PaCO_2$ > 60 mmHg), especially after muscle relaxation and prone ventilation, it is necessary to consider to implement ECLS. However, it is suggested that ECLS treatment should only be carried out under the condition that the professional center is with access to expertise. Currently the ECLS in ICU includes VV-ECMO (blood is pumped from femoral vein, and returns to right atrium through internal jugular vein after oxygenation through membrane oxygenator) and VA-ECMO (blood is pumped from femoral vein and directly enters aortic system through femoral artery after oxygenation through membrane oxygenator). For patients with severe refractory hypoxemia, neuromuscular blockade can improve oxygen supply, especially if there is still evidence of ventilator-patient dyssynchrony after the use of sedatives. However, neuromuscular blockade through continuous infusion should not be routinely used in patients with moderate-severe ARDS; Where available, ECMO in conjunction with low tidal-volume mechanical

ventilation can be considered in the treatment of patients with severe refractory hypoxemia in whom standard therapy are failing; Routine use of high-frequency oscillatory ventilation (HFOV) in patients with moderate-severe ARDS is not beneficial, but may be harmful. However, HFOV may still be regarded as a rescue therapy for patients with severe ARDS and refractory hypoxemia. ECMO can be used in some severe ARDS patients (lung injury score > 3 or pH < 7.2 due to uncompensated hypercapnia), but it is not recommended for all ARDS patients. It can be considered to use extracorporeal carbon dioxide removal for ARDS patients, if there is more supportive research evidence in the future.

Conservative fluid management can be adopted for ARDS patients without tissue hypoperfusion. Use vasoactive drugs to improve microcirculation. Empirical antibiotics targeting the suspected potential infection should be used as soon as possible, blind or improper combination of broad-spectrum antibiotics should be avoided. Unless for special reasons, the routine use of corticosteroids should be avoided. Glucocorticoids can be used in a short time (3–5 days) according to the degree of dyspnea and the progress of chest imaging if appropriate and the recommended dose is not more than the equivalent to 1-2 mg/kg methylprednisone per day. Provide intensive standard supportive care to the critically ill patients, including prevention of deep vein thrombosis and stress-induced gastrointestinal bleeding, blood glucose control and so on. Enteral nutrition can be provided. Supplemental nutrition with omega-3 fatty acids and antioxidants is not recommended. Inhaled or intravenous beta-adrenergic agonists are not recommended to promote alveolar fluid clearance and resolution of pulmonary edema.

6.5.2 Treatment of septic shock

(1) Recognize the septic shock. When infection is suspected or confirmed, and on the basis of full fluid resuscitation, vasoconstrictor drugs are still needed to maintain mean arterial pressure (MAP) ≥65 mmHg with lactate ≥2 mmol/L, the existence of septic shock should be considered. If lactate cannot be monitored for some reasons, the following three manifestations (changes in mental state, oliguria, poor peripheral perfusion and prolonged capillary filling time) should be considered as signs of a combination of infection and hypoperfusion.
(2) In resuscitation from septic shock in adults, at least 30 ml/kg of isotonic crystalloid was considered for adults in the first 3 h. In resuscitation from septic shock in children, give 20 ml/kg as a rapid bolus and up to 40–60 ml/kg in first aid.
(3) Isosmotic crystal solution is recommended for resuscitation. Do not use hypotonic crystalloids, starches, or gelatins for resuscitation in the first hour. Albumin may be considered as a resuscitation fluid, but this recommendation was based on low- quality evidence under certain conditions.
(4) Administer vasoconstrictor is suggested when shock persists after fluid resuscitation, noradrenaline as the first choice. The initial blood pressure target is MAP ≥65 mmHg in adults and age-appropriate targets in children.
(5) If it is not possible to place a central venous catheter, vasopressors can be infused through the peripheral vein through large vein and signs of extravasation and local tissue necrosis should be closely monitored.
(6) If extravasation occurs, stop infusion. Vasopressors can also be administered via intraosseous needles.

6.6 Condition evaluation and treatment effect evaluation

6.6.1 Criteria to withdraw ECLS

(1) Remove VV-ECMO. The oxygen concentration of the ECMO air-oxygen mixer has dropped to 21%, the air flow rate has dropped to 0, and the ventilator is not strong enough. Lasting for 2–3 h, the respiratory rate is within 25 breaths/min, SpO_2 > 92%, $PaCO_2$ is normal, and withdrawal from VV-ECMO may be considered.
(2) Remove VA-ECMO. The blood flow rate is reduced to the rate of (0.2 to 0.5 L / min) every 5 to 6 h from 3 L/min, and the hemodynamic condition is stable. The blood flow rate is reduced to 1.5 L/min within 24 h. If there is a bridging tube, the arteriovenous end can be connected with a bridging tube to form an ECMO circuit for self-circulation, so that the body's hemodynamics is driven by the heart. If hemodynamics is stable for at least 6 h, consider removing the machine.

6.6.2 Criteria for removing invasive breathing

When the patient is well aware, cough reflexes are obvious when sucking the sputum, the hemodynamics is stable, and the ventilator parameters are close to offline parameters, the spontaneous breathing test (SBT) is performed. After the SBT is passed, invasive breathing can be considered to remove the endotracheal tube.

6.6.3 Standards of transfer out of ICU

Patients do not need advanced respiratory support (HFNO, NIV, MV, ECLS, etc.); stable hemodynamics and tissue perfusion; no significant impairment of organ

function; and no need for organ support treatment (CRRT, artificial liver, etc.). Consider transferring the patient out of ICU procedure.

6.7 Discharge standards

The body temperature returned to normal for more than 3 days; respiratory symptoms improved significantly; inflammation of the lungs showed obvious signs of absorption; and respiratory nucleic acid was negative for two consecutive times (one-day sampling time interval at least); and the patient can be released from isolation.

7 Prevent and control nosocomial infection

7.1 Restriction and isolation guidelines for patient/suspected patients

See Table 7. *(Strong recommendation).*

7.2 Personal protection guidelines

According to the principles of standard prevention and tertiary protection, all personnel entering various zones should be evaluated using individual inventory tables according to the exposure risk level. Chose personal protective equipment of various levels is necessary. Personal protective equipment should be worn strictly in accordance with the instructions and only used for one time (Table 8, *Strong recommendation*).

8 Disease nursing

8.1 Nursing of isolated patients at home

The patient's home isolation scheme is shown in Table 5.

Patients should monitor their body temperature and illness at home. If your body temperature continues to be higher than 38 ℃, or your breath is getting worse, you should seek medical treatment timely.

In addition to taking protective measures, the home caregivers also should monitor their body temperature closely.

8.2 Nursing the patients

8.2.1 Nursing of oxygen therapy

Mild patients generally use a nasal catheter and a mask for oxygen. Adjust the oxygen flow as appropriate according to the patient's condition and doctor's instruction, and

Table 7 Restriction and isolation guidelines checklist for patients/suspected cases *(Strong recommendation)*

Category	Tactics	Precautions in practice
Environmental requirements	1.There should be clean areas, potentially contaminated areas, contaminated areas, contaminated channels and clean channels	1.1 clearly arrange and mark the 3 areas and transport materials or move from clean area to contaminated area. Retrograde is not allowed.
		1.2 Each area should be physically partitioned and clearly marked
	2. Isolation in single (priority strategy) Collective isolation for the confirmed patients, collective isolation for the suspected cases (alternative strategy)	2.1 < 4 persons per isolation ward, bed spacing ≥1.1 m
		2.2 Equipped with separate toilet
		2.3 Equipped with hand-cleaning and disinfection apparatus
		2.4 Minimize the unnecessary items (e.g. remove the curtains, etc.)
	3. Ensure that the environment and articles are clean and disinfected	3.1 Follow the Disinfection Guidelines checklist
		3.2 Exclusive use of articles in isolation areas
	4. Proper medical waste management	4.1 The medical waste should be put in sealed double-layer yellow medical waste bags for regulated disposal procedure.
Requirements to the patient/suspected Patient	5. Restrict the range of patient/suspected patient for their activities.	5.1 No escort or minimize the number of escorts.
		5.2 Clear route for patient transport (get in or out through contaminated channels)
		5.3 Patients going out should wear N95 masks or surgical masks
		5.4 Follow the disinfection guidelines after being discharged from hospital.
Requirements to the medical staff request	6. Medical personnel enter the isolation area with proper self-protection through designated channels.	6.1 Medical staff should perform the personal protection practice under the Personal Protection Guidelines in Table 8

Table 8 Personal protection guidelines checklist *(Strong recommendation)*

Item	Exposure intensity of infection risk[a]	Protective measurement								
		Round hat	N95 mask	Coverall	Eye protector/ Protective panel	Latex gloves	Barrier gown	Protective clothing	Shoe cover/ Bootstrap	Comprehensive respiratory apparatus
Recommendations as per work area										
Pre-examination triage	Low	✓	✓	✓		✓	✓			
General out-patient service	Low	✓	✓	✓		✓				
General ward	Medium	✓	✓	✓	✓	✓	✓			
	High	✓	✓	✓	✓	✓	✓	✓		
Fever clinic	Medium	✓	✓	✓	✓	✓	✓	✓	✓	
	High	✓	✓	✓	✓	✓	✓	✓	✓	✓
Isolation room (Area)	Medium	✓	✓	✓	✓	✓	✓	✓	✓	
	High	✓	✓	✓	✓	✓	✓	✓	✓	✓
Department of infectious diseases	Medium	✓	✓	✓	✓	✓	✓	✓	✓	
	High	✓	✓	✓	✓	✓	✓	✓	✓	✓
Recommendations as per personnel										
Medical staff in the isolation area	High	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Medium	✓	✓	✓	✓	✓	✓	✓	✓	
Staff in pre-examination triage	Medium	✓	✓	✓	✓	✓	✓	✓	✓	
Medical staff in Out-patient Department	Medium	✓	✓	✓	✓	✓	✓	✓	✓	
Medical staff in the observing ward	High	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Medium	✓	✓	✓	✓	✓	✓	✓	✓	
Assisting staff	Medium	✓	✓	✓	✓	✓	✓			
Administrative and supporting staff	Low	✓	✓	✓	✓	✓	✓			

[a]Low risk, general contact with patients or exposure to contaminated environment, such as escorting the patients during diagnosis, triage, palpation, consultation, etc
Medium risk, direct contact with body fluid, mucosa or incomplete skin, such as oral examination, puncture, oral care, surgery, etc
High risk, there is a risk of spatter of secretions or contaminants onto the body and face of medical staff, such as oral diagnosis, endotracheal intubation, etc

closely monitor the patient's breathing and blood oxygen saturation. If oxygen therapy fails to reach the expected effect, the nurse should analyze the cause comprehensively and be vigilant to notify the doctor.

8.2.2 Nursing of medication

Mild patients generally use antiviral drugs, antibacterial drugs (when bacterial infection exists), and symptomatic treatment. The doctor's advice should be followed accurately and timely. The adverse reactions of oseltamivir mainly include nausea, vomiting, diarrhea, abdominal pain and bronchitis, cough, etc. The adverse reactions of interferon are mainly flu-like symptoms such as fever, fatigue, myalgia, and headache, followed by mild suppression of bone marrow. Attention should be paid to identify the change of clinical manifestations or adverse drug reactions.

8.2.3 Nutritional support

According to the patients' condition, provide high-protein, high-vitamin, carbohydrate-containing diets (e.g. eggs, fish, lean meat, milk, etc.) for enough nutrition to improve physical condition.

8.2.4 Psychological nursing

Take good care of the patient and respond to the patient's question timely. Positively encourage patients to reduce their anxiety and fear.

8.3 Nursing of critically illed patients

8.3.1 Condition monitoring

Dynamically monitor patients' vital signs, water-electrolytes balance, acid-base balance, and functions of various organs, monitor patients' infection indicators, and determine the occurrence of complications such as acute respiratory distress syndrome, septic shock, stress ulcers, and deep vein thrombosis.

8.3.2 Sequential oxygen care

The critically illed patients mainly use oxygen therapy such as HFNO, NIV and invasive mechanical ventilation.

When using various oxygen treatments in a sequential manner, the airway and breathing circuit need to be kept open, and the effect of oxygen treatment needs to be monitored dynamically. At the same time, skincare products need to be used reasonably to avoid damage to the nose, face and lips by pressure. When using a high-flow nasal catheter to inhale oxygen, the oxygen flow and temperature and humidity should be adjusted appropriately. When using non-invasive mechanical ventilation, patient should receive relevant health education. Patients are instructed to inhale through the nose. The pressure is set from low to high and gradually reaches the target value. The human-machine coordination is maximized. The patient's consciousness and respiratory function are closely observed. Patients with artificial airway established should use a closed suction tube to reduce virus spread. Nurses should wear goggles or a face shield to avoid occupational exposure.

8.3.3 Special treatment nursing

If the patient develops moderate to severe ARDS, invasive mechanical ventilation combined with a prone position need to be adopted. Standard operating procedure for prone position needs to be followed. At the same time, be cautious to prevent pressure ulcers, falling bed, tube slippage, and eye damage by pressure and other complications. Patients treated with ECMO should be monitored for the performance of the oxygenator. If the oxygenator changes its color to darker, indicating the possibility of coagulation, the doctor should be notified to adjust the heparin dose as necessary. The oxygenator should be replaced if necessary. The coagulation function need to be monitored dynamically, including the whole set of coagulation and DIC (disseminated intravascular coagulation), and the time of activating partial thromboplastin, etc., the patient should be closely observed for signs of bleeding, such as bruising on the skin and mucous membranes, bleeding in the nasal cavity, oral cavity, bloody sputum, hematuria, blood in the stool, swelling of the abdomen, moving dullness, and the size of bilateral pupils. Make sure that the ECMO pipelines are tightly connected and firmly fixed to prevent air embolism and pipeline slippage.

8.3.4 Infection prevention

Perform oral care and skin care, assist the patient to use toilet, and take eyes on the indwelling tubes. Rules and regulations for aseptic operation and isolation should be strictly followed to prevent ventilator-related pneumonia, catheter-related sepsis, urinary catheter related urinary tract infections and other secondary infections.

8.3.5 Nutrition support

Dynamically assess the patients' nutritional risks and timely nutritional support can be given if needed. For the patients who can eat, the diet rich in protein and carbohydrates is recommended. Those patients who cannot eat but are compatible with enteral nutrition should be given enteral nutrition as soon as possible. For the patients incompatible with enteral nutrition, parenteral nutrition should be given timely to meet energy requirement.

8.3.6 Psychological nursing

Psychological and humanistic care should be performed in high priority especially for the awake patients. Psychological techniques like mindfulness - based stress reduction can be adopted to relieve the patients' anxiety and panic by building up their optimistic confidence in overcoming the disease.

9 Limitations of this guideline

Our guideline has three major limitations: Firstly, time is so limited that we cannot fully consider all clinical issues for this emergency disease. Secondly, many evidences came from data search is indirect. Thirdly, because some recommendations are based on the evidence from existing guidelines and experts' experience, there are situations where strong recommendations were produced on the base of low-quality evidence or very low-quality evidence, so high-quality evidence, when they appear, is likely to change current recommendations.

10 Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40779-020-0233-6.

Additional file 1. A successful treatment case of the severe 2019-nCoV infected pneumonia patient.

Additional file 2. Experience and lessons in hospital rescue for 2019-nCoV infections.

Abbreviations

2019-nCoV: 2019 novel coronavirus; ALT: Alanine aminotransferase; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; CCEBTCM: China Center for Evidence Based Traditional Chinese Medicine; CDC: Centers for Disease Control and Prevention; CK: Creatine kinase; CPAM: China International Exchange and Promotive Association for Medical and Health Care; CPAP: Continuous positive airway pressure; CRP: C-reactive protein; CRRT: Continuous renal replacement therapies; DIC: Disseminated intravascular coagulation; ECLS: Extracorporeal life support; ECMO: Extracorporeal membrane oxygenation; ESR: Erythrocyte sedimentation rate; GRADE: Grading of Recommendations Assessment, Development and Evaluation; HFNO: High flow nasal oxygen therapy; HFOV: High-frequency oscillatory ventilation; HRCT: High-resolution computed tomography; ICU: Intensive Care Unit; IL: Interleukin; MAP: Mean arterial pressure; MERS: Middle East respiratory syndrome; NGS: Next generation sequencing; NICE: National Institute for Health and Clinical Excellence; NIV: Non-invasive ventilation; PCT: Procalcitonin; PEEP: Positive end-expiratory pressure; PLT: Platelet; RCTs: Randomized controlled trials; SARS: Severe acute respiratory syndrome; SBT: Spontaneous breathing test;

TB: tuberculosis; TNF: Tumor Necrosis Factor; WBC: White blood cells; WHO: World Health Organization

Acknowledgements
The authors thank all the staff of the Novel Coronavirus Management and Research Team in Zhongnan Hospital of Wuhan University, all the authors for their hard work for the included evidences in this guideline, and the staff of the Military Medical Research editorial office for their hard work. We also thank Hao Chen's help, the college of Acupuncture and Chinese Tuina, Nanjing University of Chinese Medicine.

Authors' contributions
WXH, WYY, HLQ, ZXT, ZY and LLK composed the Consultant/Advisor's panel. CL, CZS, CH, FYP, HB, HF, LYR, LK, MJ, PYB, PZY, PZY, SHM, WCJ, WDF, XJ, XY, XHB, YYF, YTS, ZXC, ZYW, ZYG, and ZHM composed the Consensus experts' panel. DT, FC, HD, HQ, LBH, LLS, MLL, WYY, WH, ZMJ and ZH composed the Evidences synthesis panel. JYH, YXM (Canada) and RXQ were the methodologists. WY and HY were the secretaries. JYH, ZXT and the members of the evidences synthesis panel drafted the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported (in part) by the Entrusted Project of National Center for Medical Service Administration, National Health and Family Planning Commission China (No. [2019]099), the First Level Funding of the Second Medical Leading Talent Project in Hubei Province, and the Special Project for Emergency of the Ministry of Science and Technology (2020YFC0841300).

Availability of data and materials
The data and materials used during the current review are all available in this review.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Written informed consent for publication of the clinical details and clinical images was obtained from all patients. A copy of the consent form is available for review by the Editor of this journal.

Competing interests
The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare that there are no conflicts of interest in this study.

Author details
[1]Center for Evidence-Based and Translational Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [2]Institute of Hospital Management, Wuhan University, Wuhan 430071, China. [3]Department of Respiratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [4]Department of Pharmacy, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [5]Institute of Evidence-Based Medicine and Knowledge Translation, Henan University, Kaifeng 475000, China. [6]China Academy of Chinese Medical Sciences, Beijing 100700, China. [7]China Center for Evidence Based Traditional Chinese Medicine (CCEBTCM), Beijing 100700, China. [8]Department of Critical Care Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [9]Department of Clinical Laboratory, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [10]Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [11]Division of Medical Affairs, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [12]Division of Nursing Affairs, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [13]Office of Nosocomial Infection Control, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [14]Emergency Center, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [15]Department of Radiology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [16]Department of Health Research Methods, Evidence, and Impact, McMaster University, Hamilton, ON L8S 4L8, Canada. [17]Department of Traditional Chinese Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. [18]Global Health Institute, Wuhan University, Wuhan 430072, China.

Received: 29 January 2020 Accepted: 30 January 2020
Published online: 06 February 2020

References

1. World Health Organization. WHO handbook for guideline development. 2nd ed; 2019. http://apps.who.int/medicinedocs/documents/s22083en/s22083en.pdf. Accessed 22 Jan 2020
2. Norris SL. WHO and rapid advice guidelines: history and future directions. 2013. https://g-i-n.net/conference/past-conferences/10th-conference/monday/10-00-am-to-1-00-pm/norris-62.pdf/view. Accessed 22 Jan 2020.
3. Schünemann HJ, Zhang Y, Oxman AD, Expert Evidence in Guidelines Group. Distinguishing opinion from evidence in guidelines. BMJ. 2019;366:l4606.
4. GRADE handbook, GRADE working group. 2013. https://gdt.gradepro.org/app/handbook/handbook.html. Accessed 22 Jan 2020.
5. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336(7650):924–6.
6. Legault K, Schunemann H, Hillis C, et al. McMaster RARE-Bestpractices clinical practice guideline on diagnosis and management of the catastrophic antiphospholipid syndrome. J Thromb Haemost. 2018;16:1656–64.
7. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (Full version). Yixue Xinzhi. 2020;30(1):35–64. https://doi.org/10.12173/j.issn.1004-5511.2020.01.09 [Article in China].
8. Chan JFW, Yuan S, Kok KH, To KKT, Chu HC, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020. https://doi.org/10.1016/S0140-6736(20)30154-9.
9. Health Emergency Office of National Health Commission of the PRC. Update on the epidemic of novel coronavirus (2019-nCoV) infected pneumonia as at 24:00 on 26 January. http://www.nhc.gov.cn/xcs/yqfkdt/202001/3882fdcdbfdc4b4fa4e3a829b62d518e.shtml. Accessed 26 Jan 2020.
10. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020. https://doi.org/10.1038/s41586-020-2012-7.
11. Perlman S. Another decade, another coronavirus. N Engl J Med. 2020. https://doi.org/10.1056/NEJMe2001126.
12. General Office of National Health Committee. Office of State Administration of Traditional Chinese Medicine. Notice on the issuance of a programme for the diagnosis and treatment of novel coronavirus (2019-nCoV) infected pneumonia (Trial Version 4). 2020. http://bgs.satcm.gov.cn/zhengcewenjian/2020-01-28/12576.html. Accessed 29 Jan 2020.
13. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020. https://doi.org/10.1056/NEJMoa2001017.
14. General Office of National Health Committee. Office of State Administration of Traditional Chinese Medicine. Notice on the issuance of a program for the diagnosis and treatment of novel coronavirus (2019-nCoV) infected pneumonia (Trial Version 3). 2020. http://www.nhc.gov.cn/xcs/zhengcwj/202001/f492c9153ea9437bb587ce2ffcbee1fa.shtml. Accessed 24 Jan 2020.
15. Information Office of the State Council. Information office to hold press conference on joint preventing and controlling the epidemic of novel coronavirus (2019-nCoV) infected pneumonia. 2020. http://www.scio.gov.cn/xwfbh/xwbfbh/wqfbh/42311/42478/index.htm. Accessed 26 Jan 2020.
16. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020. https://doi.org/10.1016/S0140-6736(20)30183-5.
17. Li L, Ren MJ, Zhang YY, Li WQ, Zhao HY, Liang LC, et al. Lung CT image of a confirmed case of the 2019 novel coronavirus (2019-nCoV) infected pneumonia (With differential diagnosis of the SARS). Yixue Xinzhi. 2020; 30(1):4–6 [Article in China].
18. Disease Control and Prevention Bureau of National Health and Health Commission. Program for prevention and control of novel coronavirus (2019-nCoV) infected pneumonia (Trial Version 2). 2020. http://www.nhc.gov.cn/xcs/zhengcwj/202001/c67cfe29ecf1470e8c7fc47d3b751e88.shtml. Accessed 23 Jan 2020.
19. World Health Organization. Home care for patients with suspected novel coronavirus (nCoV) infection presenting with mild symptoms and management of contacts. 2020. https://www.who.int/publications-detail/home-care-for-patients-with-suspected-novel-coronavirus-(ncov)-infection-presenting-with-mild-symptoms-and-management-of-contacts. Accessed 24 Jan 2020.

20. Kim JY, Song JY, Yoon YK, Choi SH, Song YG, Kim SR, et al. Middle East respiratory syndrome infection control and prevention guideline for healthcare facilities. Infect Chemother. 2015;47(4):278–302.
21. Wang Y, Lin LK. An advice guideline recommended by central south hospital for the suspected patients of novel coronavirus (2019-nCoV) infected pneumonia and their close contactsas at home quarantine. 2020. https://mp.weixin.qq.com/s/xFO10WAFB9OUnM7VN92R2w. Accessed 25 Jan 2020.
22. World Health Organization. Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected. 2020. https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(ncov)-infection-is-suspected-20200125. Accessed 26 Jan 2020.
23. World Health Organization. WHO advice for international travel and trade in relation to the outbreak of pneumonia caused by a new coronavirus in China. 2020. https://www.who.int/ith/2020-0901_outbreak_of_Pneumonia_caused_by_a_new_coronavirus_in_C/en/. Accessed 23 Jan 2020.
24. Centers for Disease Control and Prevention. CDC: travelers' health - novel coronavirus in China. 2020. https://wwwnc.cdc.gov/travel/notices/watch/novel-coronavirus-china. Accessed 23 Jan 2020.
25. Wang W, Hu H, Song L, Gong X, Qu Y, Lu Z. Image of pulmonary and diagnosis of atypicalnovel coronavirus (2019-nCoV) infected pneumonia: case series of 14 patients. Yixue Xinzhi. 2020;30(1):7–9 [Article in China].
26. Chan KS, Lai ST, Chu CM, Tsui E, Tam CY, Wong MM, et al. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study. Hong Kong Med J. 2003;9(6):399–406.
27. Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax. 2004;59(3):252–6.
28. Lai ST. Treatment of severe acute respiratory syndrome. Eur J Clin Microbiol Infect Dis. 2005;24(9):583–91.
29. Brigitte M, Arti B, Stephan A, Judith A, Bruno R, Christoph W, et al. MERS-COV disease associated ARDS -a case report. Crit Care Med. 2015;43(12):308.
30. Jiang H, Deng H, Wang Y, Liu Z, Sun WM. The possibility of using Lopinave/Litonawe (LPV/r) as treatment for novel coronavirus 2019-nCov pneumonia: a quick systematic review based on earlier coronavirus clinical studies. Zhonghua Jizhen Yixue Zazhi. 2020;29(2):182–6 [Article in China].
31. Falzarano D, de Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, et al. Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. Nat Med. 2013;19(10):1313–7.
32. Chan JF, Yao Y, Yeung ML, Deng W, Bao L, Jia L, et al. Treatment with lopinavir/ritonavir or interferon-β1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset. J Infect Dis. 2015;212(12):1904–13.
33. Omrani AS, Saad MM, Baig K, Bahloul A, Abdul-Matin M, Alaidaroos AY, et al. Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study. Lancet Infect Dis. 2014;14(11):1090–5.
34. Khan PA, Nousheen BB, Maryam N, Sultana K. Middle east respiratory syndrome (MERS): a systematic review. Int J Pharm Sci Res. 2018;9(7):2616–25.
35. Zhao ZW, Zhang FC, Xu M, Huang K, Zhong WN, Cai WP, et al. Clinical analysis of 190 cases of outbreak with atypical pneumonia in Guangzhou in spring, 2003. Zhonghua Yixue Zazhi. 2003;83(9):713–8.
36. Meng QH, Dong PL, Guo YB, Zhang K, Liang LC, Hou W, et al. Use of glucocorticoid in treatment of severe acute respiratory syndrome cases. Zhonghua Yufang Yixue Zazhi. 2003;37(4):233–5 [Article in China].
37. Xiao JZ, Ma L, Gao J, Yang ZJ, Xing XY, Zhao HC, et al. Glucocorticoid-induced diabetes in severe acute respiratory syndrome: the impact of high dosage and duration of methylprednisolone therapy. Zhonghua Neike Zazhi. 2004;43(3):179–82 [Article in China].
38. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012;307(23):2526–33.

COVID-19: sorveglianza attiva conditio sine qua non per bloccare la trasmissione sostenuta

Fin dalle prime settimane dall'esordio dell'epidemia di SARS-COV-2, vi è stata la produzione di numerosi modelli matematici per stimarne evoluzione e prospettive.
Ovviamente, anche la più raffinata attività di *mathematical modelling* risente, in tali contesti, di limitazioni quali la relativa paucità dati e la necessità dunque di fare molte assunzioni quali premesse. Ciò spiega anche le diverse stime tra modelli differenti, sulla base appunto di diversi tipi di assunzioni *ab initio*.
È stato comunque chiaro fin da subito che solo un'attività di intensa sorveglianza attiva, per individuare i casi prima che sviluppassero sintomi degni di nota e/o richiedenti ospedalizzazione, potesse ridurre il rischio di diffusione del contagio.

Riferimento bibliografico

Thompson RN. Novel Coronavirus Outbreak in Wuhan, China, 2020: Intense Surveillance Is Vital for Preventing Sustained Transmission in New Locations. J. Clin. Med. 2020, 9: 498.

Segue articolo full text originale

Journal of
Clinical Medicine

MDPI

Article

Novel Coronavirus Outbreak in Wuhan, China, 2020: Intense Surveillance Is Vital for Preventing Sustained Transmission in New Locations

Robin N. Thompson [1,2]

[1] Mathematical Institute, University of Oxford, Woodstock Road, Oxford OX2 6GG, UK; robin.thompson@chch.ox.ac.uk
[2] Christ Church, University of Oxford, St Aldates, Oxford OX1 1DP, UK

Received: 25 January 2020; Accepted: 7 February 2020; Published: 11 February 2020

Abstract: The outbreak of pneumonia originating in Wuhan, China, has generated 24,500 confirmed cases, including 492 deaths, as of 5 February 2020. The virus (2019-nCoV) has spread elsewhere in China and to 24 countries, including South Korea, Thailand, Japan and USA. Fortunately, there has only been limited human-to-human transmission outside of China. Here, we assess the risk of sustained transmission whenever the coronavirus arrives in other countries. Data describing the times from symptom onset to hospitalisation for 47 patients infected early in the current outbreak are used to generate an estimate for the probability that an imported case is followed by sustained human-to-human transmission. Under the assumptions that the imported case is representative of the patients in China, and that the 2019-nCoV is similarly transmissible to the SARS coronavirus, the probability that an imported case is followed by sustained human-to-human transmission is 0.41 (credible interval [0.27, 0.55]). However, if the mean time from symptom onset to hospitalisation can be halved by intense surveillance, then the probability that an imported case leads to sustained transmission is only 0.012 (credible interval [0, 0.099]). This emphasises the importance of current surveillance efforts in countries around the world, to ensure that the ongoing outbreak will not become a global pandemic.

Keywords: 2019-nCoV; mathematical modelling; infectious disease epidemiology; major outbreak; forecasting; coronavirus; Wuhan; SARS

1. Introduction

The infectious agent driving the ongoing pneumonia outbreak (the 2019-nCoV) appears to have transitioned from animals into humans, with the Huanan seafood wholesale market in Wuhan, China, representing the most likely source [1–5]. Since then, cases have been recorded in other countries, and initial estimates suggest a hospital fatality risk of around 14% [6], although estimates of disease severity early in an outbreak are often imprecise [7–9]. Even countries without confirmed cases have been on high alert. For example, even prior to the two cases in the United Kingdom on 31 January 2020, officials were reported to be attempting to trace as many as 2000 visitors that had travelled to that country from Wuhan [10].

The most devastating infectious disease outbreaks are those that have a wide geographical range, as opposed to being confined to a small region [11,12]. The previously known virus that is most similar to the 2019-nCoV is the SARS coronavirus [13], which generated cases in 37 countries in 2002–2003 [13,14]. Since the 2019-nCoV is clearly capable of being transmitted by infected hosts to countries around the world, an important question for policy makers is whether or not these imported cases have the potential to generate sustained human-to-human transmission in new locations.

Here, we present data describing the times from symptom onset to hospitalisation for 47 patients from the current outbreak, obtained from publicly available line lists [15]. We fit an exponential distribution to these data, accounting for uncertainty due to the limited numbers of patients from whom data were available. Assuming that this distribution characterises the time spent by infected hosts generating new transmissions in the community, it is then possible to infer the probability that a case arriving in a new location is followed by an outbreak driven by sustained human-to-human transmission. We estimate this probability under the assumption that the transmissibility of the 2019-nCoV is similar to that of the SARS coronavirus, and then go on to consider the effect of shortening the mean time from symptom onset to hospitalisation. This provides an estimate of the risk that imported cases generate sustained outbreaks in new locations under different surveillance levels.

2. Methods

2.1. Time from Symptom Onset to Hospitalisation

The distribution of times from symptom onset to hospitalisation was estimated using patient data from the ongoing outbreak [15] (data are shown in Figure 1A). Since the precise times of symptom onset and hospitalisation on the dates concerned were unknown, we converted the times from symptom onset to hospitalisation to intervals describing possible time periods (see the Supplementary Data). For example, for a case showing symptoms on 9 January 2020, and then being hospitalised on 14 January 2020, the time between symptom onset and hospitalisation lies between four and six days (see e.g., Supplementary Material of [16] for a similar calculation). This is because the minimum possible period involves symptom onset at the end of 9 January and hospitalisation at the start of 14 January, whereas the maximum possible period involves symptom onset early on 9 January and hospitalisation late on 14 January.

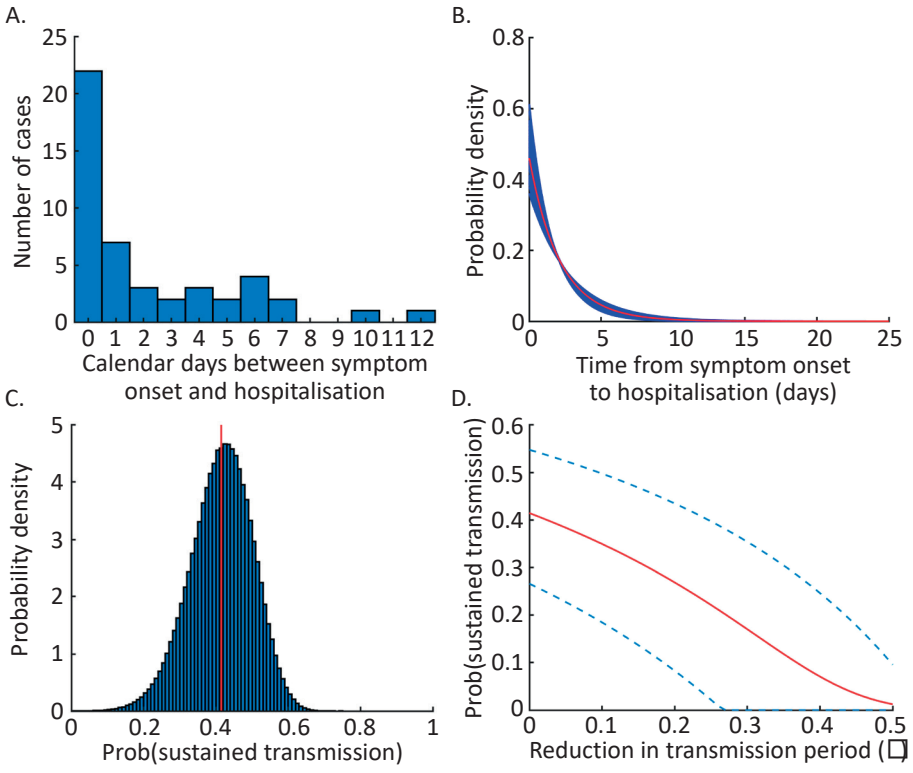


Figure 1. The probability of a self-sustaining outbreak driven by human-to-human transmission arising following the importation of one infected individual. (**A**) Data describing the number of days between symptom onset and hospitalisation for 47 patients in the ongoing outbreak [15]. (**B**) The

estimated distribution of times between symptom onset and hospitalisation, obtained by fitting to the data shown in panel A. Blue lines show a range of equally possible distributions (see Methods; 50 distributions are shown here, selected at random from the n = 100,000 distributions considered), and the red line shows the average of the n = 100,000 distributions. (**C**) The probability of sustained transmission for each possible distribution of times from symptom onset to hospitalisation (Equation (1); blue histogram) and the probability of sustained transmission obtained by integrating over the possible distributions (Equation (2); red line). (**D**) The probability that a single imported case leads to sustained transmission in a new location, for different surveillance levels. The red line shows the mean estimates (obtained using Equation (2) but extended to account for intensified surveillance), and the blue dotted lines show the 5th and 95th percentile estimates (obtained when Equation (1) is applied, but extended to account for intensified surveillance).

We then fitted the rate parameter (γ) of an exponential distribution to these interval-censored data using Markov chain Monte Carlo (MCMC). A chain of length 10,000,000 in addition to a burn-in of 100,000 was used. The chain was then sampled with a thinning interval of 100 steps, giving rise n = 100,000 equally possible distributions for the times from symptom onset to hospitalisation, each characterised by a parameter estimate γ_i $(i = 1,2,\ldots,n)$. For further details of the MCMC algorithm used, see the Supplementary Text.

2.2. Estimating the Probability of Sustained Transmission

The distributions of times from symptom onset to hospitalisation were used to estimate the probability that an imported case will lead to sustained transmission, by assuming that infections occur according to a branching process (e.g., [17–19]). In this branching process, the effective reproduction number (accounting for control interventions, other than intensified surveillance which we model explicitly) of the 2019-nCoV when the virus arrives in a new location is denoted by $R = \beta/\gamma$, where the parameter β represents pathogen transmissibility [20]. We assumed that the transmissibility of the virus is similar to that of the SARS coronavirus, i.e., $\beta = R_{SARS}\gamma_{SARS}$, where $R_{SARS} = 3$ [21] and the mean infection duration for SARS is $1/\gamma_{SARS} = 3.8$ days [22]. However, we adjusted the infectious period to account for the data describing the times between symptom onset and hospitalisation in the current outbreak. In doing this, we assumed that the time between an individual first displaying symptoms and being hospitalised was the period in which that individual was potentially transmitting the virus in the community.

The probability of a sustained transmission chain [19,20] starting from a single index case can be estimated for each of the equally possible distributions for the time from symptom onset to hospitalisation,

$$\text{Prob}(\text{sustained transmission} \mid \gamma_i) = 1 - \frac{1}{(\beta/\gamma_i)} \quad (1)$$

In this expression, it is assumed that $\beta/\gamma_i > 1$ (otherwise the probability of sustained transmission takes the value zero). If required, this can then be combined into a single estimate for the probability that an imported case leads to sustained transmission, p, given by

$$p = \frac{1}{n}\sum_{i=1}^{n} \text{Prob}(\text{sustained transmission} \mid \gamma_i) \quad (2)$$

To include intensified surveillance in these estimates, we simply replaced the mean time from symptom onset to hospitalisation for each of the equally plausible distributions, $1/\gamma_i$, by the modified expression $(1-\rho)/\gamma_i$. In this approximation, the parameter ρ represents the proportional reduction in the mean infectious period due to intensified surveillance.

2.3. Multiple Imported Cases

The risk of sustained transmission given multiple imported cases was calculated by considering the possibility that none of those cases led to sustained transmission. Consequently,

$$\text{Prob(sustained transmission} \mid m \text{ imported cases, } \gamma_i) = 1 - \left(\frac{1}{(\beta(1-\rho)/\gamma_i)}\right)^m \quad (3)$$

In this expression, it is assumed that $\beta(1-\rho)/\gamma_i > 1$ (otherwise the probability of sustained transmission takes the value zero). Again, if required, this can be combined into a single estimate for the probability of sustained transmission starting from m imported cases, p_m, given by

$$p_m = \frac{1}{n}\sum_{i=1}^{n} \text{Prob(sustained transmission} \mid m \text{ imported cases, } \gamma_i) \quad (4)$$

3. Results

As described in Methods, the distribution of times between symptom onset and hospitalisation was estimated using Markov chain Monte Carlo (Figure 1B and Figure S1) from the patient data in Figure 1A. This gave rise to a range of equally plausible distributions describing these time periods (blue lines in Figure 1B). The average of these distributions is shown by the red line in Figure 1B, however we used the full range of distributions in our calculations of the probability of sustained transmission occurring from each imported case.

Each of the range of plausible distributions corresponded to an estimate for the probability of a self-sustaining outbreak (Equation (1) and histogram in Figure 1C). A single estimate for the probability of sustained transmission can be obtained by summing over the range of distributions using Equation (2). The resulting probability of sustained transmission is 0.41 (red line in Figure 1C), with credible interval (CrI) [0.27, 0.55], where the CrI reflects the 5th and 95th percentile estimates.

We then considered the reduction in the probability that an imported case leads to sustained transmission if surveillance is more intense. Specifically, we assumed that intensified surveillance led to a reduction in the mean period from symptom onset to hospitalisation, governed by the parameter ρ (where $\rho = 0$ corresponds to no intensification of surveillance, and $\rho = 1$ corresponds to an implausible scenario in which infectious cases are hospitalised immediately). We found that, if surveillance is intensified so that the mean time from symptom onset to hospitalisation is halved, the probability that each imported case leads to sustained transmission is reduced to only 0.012 (CrI [0, 0.099]; Figure 1D).

Finally, we considered the combined effect if multiple cases arrive in a new location. In that scenario, intense surveillance has the potential to reduce the risk of sustained transmission significantly compared to weak surveillance. For ρ = 0.5, the probability that any of 10 imported cases generate a substantial outbreak is only 0.12 (CrI [0, 0.65]; Figure 2C). This highlights the importance of rigorous surveillance, particularly in locations where infected hosts are most likely to travel.

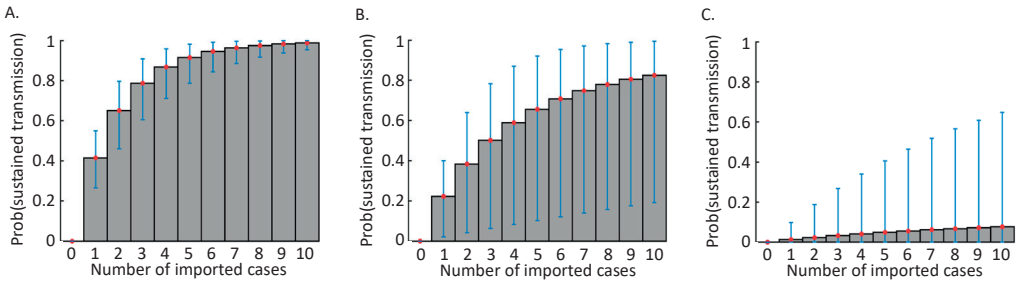


Figure 2. The probability of a self-sustaining outbreak driven by human-to-human transmission arising from multiple independent cases imported to a new location, under different surveillance levels. (**A**) No intensification of surveillance ($\rho = 0$). (**B**) Medium level of surveillance intensification ($\rho = 0.25$). (**C**) High level of surveillance intensification ($\rho = 0.5$). The grey bars and red dots show the mean estimates (obtained using Equation (4)), and the error bars indicate the 5th and 95th percentile estimates obtained when Equation (3) is applied.

4. Discussion

There are concerns that the ongoing outbreak driven by the 2019-nCoV could spread globally [3,5,23–25] with sustained transmission in countries around the world. Periods of high travel rates, such as the recent Chinese New Year holiday, present a significant challenge since they pose an elevated risk of importations of the virus to new locations [3,13]. In an effort to prevent a surge in travel, the Chinese government extended the national New Year holiday in January 2020.

Here, we have estimated the potential for cases arriving in new locations to lead to sustained transmission. According to the basic model that we have constructed, if surveillance levels are similar to those in China at the beginning of the current outbreak, and if this virus is similarly transmissible to the SARS coronavirus that spread globally in 2002–2003, then the probability that each imported infected case generates an outbreak due to sustained transmission is approximately 0.41 (CrI [0.27, 0.55]; Figure 1C). However, under a higher level of surveillance, the risk of sustained outbreaks is substantially lower (Figure 1D). This result is particularly striking when multiple independent cases travel to a new location, either simultaneously or in sequence (Figure 2). In that scenario, intensified surveillance is particularly important.

Our study involves the simplest possible model that permits the risk of sustained transmission to be estimated from the very limited data that have been collected in this outbreak until now. As additional information becomes available, it will be possible to estimate the risk of outbreaks in new locations with more precision. We made the assumption that symptom appearance coincides with the onset of infectiousness. One of the features of the SARS outbreak in 2002–2003 that allowed it to eventually be brought under control was the low proportion of onward transmissions occurring either prior to symptoms or from asymptomatic infectious hosts [26]. It might be hoped that infections due to the 2019-nCoV share this characteristic. Some reports have suggested that this may not be the case, although the extent of presymptomatic transmission is disputed [25,27]. We are working on an updated version of our analyses that includes the possibility of transmission from presymptomatic or mildly symptomatic hosts (based on the "SEAIR" compartmental model [19]).

Since our results were obtained using patient data from early in the ongoing outbreak, surveillance systems may not have been fully established when these data were collected, and patients may not have been primed to respond quickly to early symptoms. Our results might therefore be viewed as an upper bound on the risk posed by the 2019-nCoV. As the outbreak continues, it might be expected that the time from symptom onset to hospitalisation or isolation will decrease, leading to lower risks of sustained transmission, as has been observed for outbreaks of other diseases (e.g., the ongoing outbreak of Ebola virus disease in the Democratic Republic of the Congo). Initial indications suggest that such a decrease is occurring in China for this outbreak. In contrast, there may be some individuals that developed symptoms, but had not yet reported their infection by the time our analysis was conducted. "Right censoring" in this way favours lower reporting times, and so falsely reduces estimates of the time between symptom onset and hospitalisation [16,28].

Going forwards, it will be possible to include additional realism in the model. One example might be to consider spatial variation in host population densities and surveillance levels, leading to spatially inhomogeneous outbreak risks. Another possibility might be to account more explicitly for heterogeneities between different infectors, either by incorporating "superspreaders" [29] in the model or by differentiating between individuals that report disease at different rates. Such heterogeneity might be expected to reduce the risk of sustained transmission (for a preliminary analysis, in which individuals can either be "fast reporters" or "slow reporters", see the Supplementary Text). It would also be possible to differentiate between mild and severe cases in the model. On the one hand, a mild case might be more likely to go unnoticed than a severe case, potentially leading to a higher outbreak risk. On the other hand, mild infections may generate fewer secondary cases than severe infections, thereby decreasing the outbreak risk. Complex interactions may therefore affect the risk of sustained transmission in an unpredictable fashion.

Despite the necessary simplifications made in this study, our analyses are sufficient to demonstrate the key principle that rigorous surveillance is important to minimise the risk of the

2019-nCoV generating large outbreaks in countries worldwide. We therefore support the ongoing work of the World Health Organization and policy makers from around the world, who are working with researchers and public health experts to manage this outbreak [2]. We also appreciate efforts to make data publicly available [15]. Careful analysis of the outbreak, as well as minimisation of transmission risk as much as possible, is of clear public health importance.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1.

Funding: This research was funded by Christ Church, Oxford, via a Junior Research Fellowship.

Acknowledgments: Thank you to Sebastian Funk, Christophe Fraser, Uri Obolski, David Steinsaltz and the anonymous reviewers for helpful comments about the original version of this manuscript.

Conflicts of Interest: There are no competing interests.

References

1. Hui, D.S.; Azhar, E.I.; Madani, T.A.; Ntoumi, F.; Kock, R.; Dar, O.; Ippolito, G.; Mchugh, T.D.; Memish, Z.A.; Drosten, C.; et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int. J. Infect. Dis.* **2020**, *91*, 264–266.
2. World Health Organization. Novel Coronavirus (2019-nCoV); Situation Report 3; 2020. Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports (accessed on 25 January 2020).
3. Imai, N.; Dorigatti, I.; Cori, A.; Riley, S.; Ferguson, N.M. Report 1: Estimating the Potential Total Number of Novel Coronavirus (2019-nCoV) Cases in Wuhan City, China. 2020. Available online: https://www.imperial.ac.uk/mrc-global-infectious-disease-analysis/news--wuhan-coronavirus/ (accessed on 23 January 2020).
4. Chan, J.F.W.; Yuan, S.; Kok, K.H.; To, K.K.W.; Chu, H.; Yang, J.; Xing, F.; Liu, J.; Chik-Yan Yip, C.; Wing-Shan Poon, R.; et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster. *Lancet* **2020**, *6736*, 1–10.
5. Nishiura, H.; Jung, S.M.; Linton, N.M.; Kinoshita, R.; Yang, Y.; Hayashi, K.; Kobayashi, T.; Yuan, B.; Akhmetzhanov, A.R. The extent of transmission of novel coronavirus in Wuhan, China, 2020. *J. Clin. Med.* **2020**, *9*, 330.
6. Wu, P.; Hao, X.; Lau, E.H.Y.; Wong, J.Y.; Leung, K.S.M.; Wu, J.T.; Cowling, B.J.; Leung, G.M. Real-time tentative assessment of the epidemiological characteristics of novel coronavirus infections in Wuhan, China, as at 22 January 2020. *Eurosurveillance* **2020**, *25*, 2000044.
7. Imai, N.; Cori, A.; Dorigatti, I.; Baguelin, M.; Donnelly, C.A.; Riley, S.; Ferguson, N.M. Report 3: Transmissibility of 2019-nCoV. 2020. Available online: https://www.imperial.ac.uk/media/imperial-college/medicine/sph/ide/gida-fellowships/Imperial-2019-nCoV-transmissibility.pdf (accessed on 26 January 2020).
8. Lipsitch, M.; Donnelly, C.A.; Fraser, C.; Blake, I.M.; Cori, A.; Dorigatti, I.; Ferguson, N.M.; Garske, T.; Mills, H.L.; Riley, S.; et al. Potential Biases in estimating absolute and relative case-fatality risks during outbreaks. *PLoS Negl. Trop. Dis.* **2015**, *16*, e0003846.
9. Ghani, A.C.; Donnelly, C.A.; Cox, D.R.; Griffin, J.T.; Fraser, C.; Lam, T.H.; Ho, L.M.; Chan, W.S.; Anderson, R.M.; Hedley, A.J.; et al. Methods for estimating the case fatality ratio for a novel, emerging infectious disease. *Am. J. Epidemiol.* **2005**, *162*, 479–486.
10. BBC. China Coronavirus: UK Tracing up to 2000 Wuhan Visitors. 2020. Available online: https://www.bbc.co.uk/news/uk-51232163 (accessed on 24 January 2020).
11. Tatem, A.J.; Rogers, D.J.; Hay, S.I. Global Transport Networks and Infectious Disease Spread. *Adv. Parasitol.* **2006**, *62*, 293–343.
12. Thompson, R.N.; Thompson, C.; Pelerman, O.; Gupta, S.; Obolski, U. Increased frequency of travel in the presence of cross-immunity may act to decrease the chance of a global pandemic. *Philos. Trans. R. Soc. B* **2019**, *374*, 20180274.
13. Cohen, J.; Normile, D. New SARS-like virus in China triggers alarm. *Science* **2020**, *367*, 234–235.
14. Parry, J. SARS virus identified, but the disease is still spreading. *Br. Med. J.* **2003**, *326*, 897.
15. Kraemer, M.; Pigott, D.; Xu, B.; Hill, S.; Gutierrez, B.; Pybus, O. Epidemiological Data from the nCoV-2019 Outbreak: Early Descriptions from Publicly Available Data. 2020. Available online:

http://virological.org/t/epidemiological-data-from-the-ncov-2019-outbreak-early-descriptions-from-publicly-available-data/ (accessed on 23 January 2020).

16. Thompson, R.N.; Stockwin, J.E.; Gaalen, R.D.; Van Polonsky, J.A.; Kamvar, Z.N.; Demarsh, P.A.; Dahlqwist, E.; Li, S.; Miguel, E.; Jombart, T.; et al. Improved inference of time-varying reproduction numbers during infectious disease outbreaks. *Epidemics* **2019**, *19*, 100356.
17. Lloyd, A.L.; Zhang, J.; Root, A.M. Stochasticity and heterogeneity in host-vector models. *J. R. Soc. Interface* **2007**, *4*, 851–863.
18. Allen, L.J.S.; Lahodny, G.E. Extinction thresholds in deterministic and stochastic epidemic models. *J. Biol. Dyn.* **2012**, *6*, 590–611.
19. Thompson, R.N.; Gilligan, C.A.; Cunniffe, N.J. Detecting presymptomatic infection is necessary to forecast major epidemics in the earliest stages of infectious disease outbreaks. *PLoS Comput. Biol.* **2016**, *12*, e1004836.
20. Thompson, R.N.; Jalava, K.; Obolski, U. Sustained transmission of Ebola in new locations: More likely than previously thought. *Lancet Infect. Dis.* **2019**, *19*, 1058–1059.
21. Wallinga. J.; Teunis, P. Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures. *Am. J. Epidemiol.* **2004**, *160*, 509–516.
22. Feng, D.; Jia, N.; Fang, L.Q.; Richardus, J.H.; Han, X.N.; Cao, W.C.; De Vlas, S.J. Duration of symptom onset to hospital admission and admission to discharge or death in SARS in mainland China: A descriptive study. *Trop. Med. Int. Heal.* **2009**, *14*, 28–35.
23. Bogoch, II.; Watts, A.; Thomas-Bachli, A.; Huber, C.; Kraemer, M.U.G.; Khan, K. Pneumonia of unknown etiology in Wuhan, China: Potential for international spread via commercial air travel. *J. Travel Med.* **2020**, *1*, taaa008.
24. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **2020**, *6736*, 1–10.
25. Thompson, R.N. Pandemic potential of 2019-nCoV. *Lancet Infect. Dis.* **2020**, *20*, 30068.
26. Fraser, C.; Riley, S.; Anderson, R.M.; Ferguson, N.M. Factors that make an infectious disease outbreak controllable. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 6146–6151.
27. Kupferschmidt, K. Study Claiming New Coronavirus Can be Transmitted by People without Symptoms Was Flawed. Available online: https://www.sciencemag.org/news/2020/02/paper-non-symptomatic-patient-transmitting-coronavirus-wrong (accessed on 4 February 2020).
28. Donnelly, C.A.; Ghani, A.C.; Leung, G.M.; Hedley, A.J.; Fraser, C.; Riley, S.; Abu-Raddad, L.J.; Ho, L.M.; Thach, T.Q.; Chau, P.; et al. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* **2003**, *361*, 1761–1766.
29. Lloyd-Smith, J.O.; Schreiber, S.J.; Kopp, P.E.; Getz, W.M. Superspreading and the effect of individual variation on disease emergence. *Nature* **2005**, *438*, 355–359.

Un vaccino per il nuovo coronavirus

L'Organizzazione Mondiale della Sanità già a fine gennaio ha dichiarato l'infezione da nuovo coronavirus quale emergenza globale.
Non essendo disponibili allo stato attuale terapie farmacologiche di comprovata efficacia, una delle strategie più attrattive al fine di contenere la diffusione globale del virus è quella di sviluppare un vaccino.
Naturalmente, così come per un antivirale, anche per questo tipo di presidio sono necessari tempi di sviluppo che, per quanto comprimibili sull'onda dell'emergenza sanitaria, non renderebbero disponibile l'eventuale arma preventiva prima di alcuni mesi, se non almeno un anno.
Le tipologie di vaccini che possono essere potenzialmente impiegate sono vaccini a virus inattivato o attenuato, vaccini a subunità oppure basati su DNA o mRNA o perfino su vettori virali.

Riferimento bibliografico

Shang W et al. The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines. npj Vaccines 2020; 5: 18.

Segue articolo full text originale

npj | Vaccines

www.nature.com/npjvaccines

COMMENT OPEN

Check for updates

The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines

Weilong Shang[1], Yi Yang[1], Yifan Rao[1] and Xiancai Rao[1]✉

The outbreak of 2019-novel coronavirus disease (COVID-19) that is caused by SARS-CoV-2 has spread rapidly in China, and has developed to be a Public Health Emergency of International Concern. However, no specific antiviral treatments or vaccines are available yet. This work aims to share strategies and candidate antigens to develop safe and effective vaccines against SARS-CoV-2.

npj Vaccines (2020)5:18; https://doi.org/10.1038/s41541-020-0170-0

An outbreak of 2019-novel coronavirus (SARS-CoV-2) that causes atypical pneumonia (COVID-19) has raged in China since mid-December 2019 and has spread to 26 countries (February 20, 2020). The epidemic was identified by the first four cases confirmed on December 29, 2019 and was traced to the Huanan Seafood Wholesale Market, Wuhan city, Hubei Province, China[1]. A total of 75,465 cases with SARS-CoV-2 infections have been confirmed up to date (February 20, 2020), and 2,236 people have died in China[2]. COVID-19 spreads rapidly by human-to-human transmission with a median incubation period of 3.0 days (range, 0 to 24.0), and the time from symptom onset to developing pneumonia is 4.0 days (range, 2.0 to 7.0)[3]. Respiratory droplets and direct contact are conventional transmission routes for SARS-CoV-2, and fecal-to-oral transmission might also have a role[3]. Fever, dry cough, and fatigue are common symptoms at onset of COVID-19[4]. Most patients have lymphopenia and bilateral ground-glass opacity changes on chest CT scans[4,5]. No specific antiviral treatments or vaccines are available because it is a new emerging viral disease. Development of SARS-CoV-2-based vaccines is urgently required.

The entire virus particle-based preparation of vaccines, including inactivated and attenuated virus vaccines is advisable, because it is based on previous studies about the prevention and control of seasonal influenza vaccines[6]. The first SARS-CoV-2 (Wuhan-Hu-1) was successfully sequenced and its genomic sequence submitted to GenBank on January 5, 2020 (Accession no. MN908947.3)[7]. Subsequently large-scale culture of SARS-CoV-2 was quickly performed, and an inactivated virus vaccine could be prepared through the employment of established physical and chemical methods such as UV light, formaldehyde, and β-propiolactone[8]. The development of attenuated-virus vaccines is also possible by carefully screening the serially propagated SARS-CoV-2 with reduced pathogenesis such as induced minimal lung injury, diminished limited neutrophil influx, and increased anti-inflammatory cytokine expressions compared with the wild-type virus[9]. Both inactivated and attenuated virus vaccines have their own disadvantages and side effects (Table 1). Alternatively, new vaccine designs based on the putative protective antigen/peptides derived from SARS-CoV-2 should be considered.

Accumulated releases of SARS-CoV-2 genomes such as GenBank accession numbers MN908947.3, MN975262.1, NC_045512.2, MN997409.1, MN985325.1, MN988669.1, MN988668.1, MN994468.1, MN994467.1, MN988713.1, and MN938384.1 facilitate the development of virus-based subunit vaccines. SARS-CoV-2, which is similar to SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), is an enveloped, single- and positive-stranded RNA virus with a genome comprising 29,891 nucleotides, which encode the 12 putative open reading frames responsible for the synthesis of viral structural and nonstructural proteins[7,10]. A mature SARS-CoV-2 has four structural proteins, namely, envelope (E), membrane (M), nucleocapsid (N), and spike (S)[10]. All these proteins may serve as antigens to stimulate neutralizing antibodies and increase $CD4^+$/$CD8^+$ T-cell responses[8,9]. However, subunit vaccines require multiple booster shots and suitable adjuvants to work, and certain subunit vaccines such as hepatitis B surface antigen, PreS1, and PreS2 may fail to yield protective response when tested clinically[11]. The DNA and mRNA vaccines that are easier to design and proceed into clinical trials very quickly remain experimental. The viral vector-based vaccines could also be quickly constructed and used without an adjuvant[12]. However, development of such vaccines might not start until antigens containing the neutralizing epitopes are identified[8].

The E and M proteins have important functions in the viral assembly of a coronavirus, and the N protein is necessary for viral RNA synthesis[13]. Deletion of E protein abrogated the virulence of CoVs, and several studies have explored the potential of recombinant SARS-CoV or MERS-CoV with a mutated E protein as live attenuated vaccines[13,14]. The M protein can augment the immune response induced by N protein DNA vaccine against SARS-CoV;[15] however, the conserved N protein across CoV families implies that it is not a suitable candidate for vaccine development, and the antibodies against the N protein of SARS-CoV-2 do not provide immunity to the infection[16]. The critical glycoprotein S of SARS-CoV-2 is responsible for virus binding and entry[16]. The S precursor protein of SARS-CoV-2 can be proteolytically cleaved into S1 (685 aa) and S2 (588 aa) subunits[10]. The S2 protein is well conserved among SARS-CoV-2 viruses and shares 99% identity with that of bat SARS-CoVs[10]. The vaccine design based on the S2 protein may boost the broad-spectrum antiviral effect and is worth testing in animal models. Antibodies against the conserved stem region of influenza hemagglutinin have been found to exhibit broadly cross-reactive immunity, but are less potent in neutralizing influenza A virus[17]. In contrast, the S1 subunit consists of the receptor-binding domain (RBD), which mediates virus entry into sensitive cells through the host angiotensin-converting enzyme 2 (ACE2) receptor[18]. The S1 protein of 2019-nCoV shares about 70% identity with that of human SARS-CoVs. The highest number of variations of amino acids in the RBD is located in the external subdomain, which is responsible for the direct interaction between virus and host receptor[10,18]. Blocking the initial entry of a

[1]Department of Microbiology, College of Basic Medical Sciences, Army Medical University (Third Military Medical University), 400038 Chongqing, China. ✉email: raoxiancai@126.com

Table 1. Advantages and disadvantages of different vaccine strategies.

Vaccine strategy	Advantages	Disadvantages	References
Inactivated virus vaccines	Easy to prepare; safety; high-titer neutralizing antibodies	Potential inappropriate for highly immunosuppressed individuals	25
Attenuated virus vaccines	Rapid development; induce high immune responses	Phenotypic or genotypic reversion possible; can still cause some disease	25
Subunit vaccines	High safety; consistent production; can induce cellular and humoral immune responses; high-titer neutralizing antibodies	High cost; lower immunogenicity; require repeated doses and adjuvants	12,14
Viral vector vaccines	Safety; induces high cellular and humoral immune responses	Possibly present pre-existing immunity	12
DNA vaccines	Easier to design; high safety; high-titer neutralizing antibodies	Lower immune responses in humans; repeated doses may cause toxicity	23
mRNA vaccines	Easier to design; high degree of adaptability; induce strong immune responses	Highly unstable under physiological conditions	23

virus is proposed as a successful strategy in controlling viral infection. Based on SARS vaccine development, most vaccine candidates target the S protein, which induces neutralizing antibody responses and stimulates a protective cellular immunity against SARS-CoVs[12]. Bukreyev et al.[19] showed that immunization of African green monkeys with the full-length S protein of SARS-CoV protects monkeys from subsequent homologous SARS-CoV challenge. Administration of SARS-CoV RBD proteins can also induce highly potent neutralizing antibodies and long-term protective immunity in animal models[20]. Thus, the generation of antibodies targeting the S1 subunit of SARS-CoV-2 would be an important preventive and treatment strategy that can be tested further in suitable models before clinical trials[10].

Vaccine delivery modality and immunization strategy are important issues to be considered for achieving effective antiviral immunity. As a cause of respiratory tract infection and as demonstrated by the findings of SARS-CoV-2 in stools[1,21], administration of vaccines by oral or aerosol routes will induce mucosal immune responses and are possible modes of SARS-CoV-2 vaccine immunization. A safe DNA vector for preparation of DNA vaccines[22], an attenuated virus strain for design of chimeric viral vaccines[23], and engineered safe bacteria for production of membrane vesicle-vaccines[24] could be explored for vaccine delivery and are worth investigating in the near future.

We can assume that virus-based vaccines should prove valuable in combatting COVID-19. In addition to the entire virus particle-associated inactivated or attenuated viral vaccines, the subunit candidates, such as S1 protein and/or the RBD element of SARS-CoV-2, are also valuable targets for vaccine design. Combining subunit vaccines with established or new adjuvants such as alum versus modern adjuvants such as the GSK AS series of adjuvants may represent a faster and safer strategy to move through early clinical development with the caveat that the protective efficacy may not be strong enough. As a result, immunizing the subunit vaccines with proper delivery platforms and immunization strategies to enhance the immune responses should be considered. We expect researchers who are racing against time will bring a new SARS-CoV-2-based vaccine from gene sequence to clinical testing in approximately 16–20 weeks.

Received: 11 February 2020; Accepted: 21 February 2020;
Published online: 06 March 2020

REFERENCES

1. Li, Q. et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa2001316 (2020).
2. National Health Commission of the People's Republic of China. *Epidemic Prevention and Control of Dynamic*. http://www.nhc.gov.cn/xcs/yqtb/202002/ac1e98495cb04d36b0d0a4e1e7fab545.shtml.
3. Guan, W. et al. Clinical characteristics of 2019 novel coronavirus infection in China. *medRxiv*. https://doi.org/10.1101/2020.02.06.20020974 (2020).
4. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. **395**, 497–506 (2020).
5. Duan, Y. N. & Qin, J. Pre- and posttreatment chest CT findings: 2019 novel coronavirus (2019-nCoV) pneumonia. *Radiology*. https://doi.org/10.1148/radiol.2020200323 (2020).
6. Grohskopf, L. A. et al. Prevention and control of seasonal influenza with vaccines: Recommendations of the Advisory Committee on Immunization Practices-United States, 2018–19 influenza season. *MMWR. Recomm. Rep.* **67**, 1–20 (2018).
7. Wu, F. et al. A new coronavirus associated with human respiratory disease in China. *Nature*. https://doi.org/10.1038/s41586-020-2008-3 (2020).
8. Jiang, S., He, Y. & Liu, S. SARS vaccine development. *Emerg. Infect. Dis.* **11**, 1016–1020 (2005).
9. Regla-Nava, J. A. et al. Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates. *J. Virol.* **89**, 3870–3887 (2015).
10. Chan, J. F. et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg. Microbes Infect.* **9**, 221–236 (2020).
11. Eyigun, C. P. et al. A comparative trial of two surface subunit recombinant hepatitis B vaccines vs a surface and PreS subunit vaccine for immunization of healthy adults. *J. Viral. Hepat.* **5**, 265–269 (1998).
12. Du, L. et al. The spike protein of SARS-CoV—a target for vaccine and therapeutic development. *Nat. Rev. Microbiol.* **7**, 226–236 (2009).
13. Schoeman, D. & Fielding, B. C. Coronavirus envelope protein: current knowledge. *Virol. J.* **16**, 69 (2019).
14. Graham, R. L., Donaldson, E. F. & Baric, R. S. A decade after SARS: strategies for controlling emerging coronaviruses. *Nat. Rev. Microbiol.* **11**, 836–848 (2013).
15. Shi, S. Q. et al. The expression of membrane protein augments the specific responses induced by SARS-CoV nucleocapsid DNA immunization. *Mol. Immunol.* **43**, 1791–1798 (2006).
16. Gralinski, L. E. & Menachery, V. D. Return of the coronavirus: 2019-nCoV. *Viruses* **12**, 135 (2020).
17. He, W., Mullarkey, C. E. & Miller, M. S. Measuring the neutralization potency of influenza A virus hemagglutinin stalk/stem-binding antibodies in polyclonal preparations by microneutralization assay. *Methods* **90**, 95–100 (2015).
18. Wan, Y., Shang, J., Graham, R., Baric, R. S. & Li, F. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. *J. Virol.* https://doi.org/10.1128/JVI.00127-20 (2020).
19. Bukreyev, A. et al. Mucosal immunisation of African green monkeys (*Cercopithecus aethiops*) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. *Lancet* **363**, 2122–2127 (2004).
20. Song, Z. et al. From SARS to MERS, thrusting coronaviruses into the spotlight. *Viruses* **11**, 59 (2019).
21. Holshue, M. L. et al. First case of 2019 novel coronavirus in the United States. *N. Engl. J. Med*. https://doi.org/10.1056/NEJMoa2001191 (2020).
22. Yang, Z. Y. et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature* **428**, 561–564 (2004).
23. Rauch, S., Jasny, E., Schmidt, K. E. & Petsch, B. New vaccine technologies to combat outbreak situations. *Front. Immunol.* **9**, 1963 (2018).
24. Yuan, J. et al. Safe staphylococcal slatform for the development of multivalent nanoscale vesicles against viral infections. *Nano Letters* **18**, 725–733 (2018).
25. Roper, R. L. & Rehm, K. E. SARS vaccines: where are we? *Expert Rev. Vaccines* **8**, 887–898 (2009).

ACKNOWLEDGEMENTS

This work was supported by the National Key Biosafety Technology Research and Development Program of China (2017YFC1200404-4), and the Biosafety Research Program of PLA (17SAZ08).

AUTHOR CONTRIBUTIONS

W.S. and Y.R. wrote the manuscript: Y.Y. and X.R. edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to X.R.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

La necessità di un approccio One Health per le zoonosi emergenti

SARS-COV-2 è solo l'ultimo in ordine temporale di una lunga serie recente di patogeni d'origine animale che hanno fatto il salto di specie verso l'uomo.
Ciò rappresenta un memento di come la salute di uomini, animali e ambienti sia strettamente interconnessa. Le alterazioni degli ecosistemi prodotte dall'azione dell'uomo sono tra i principali fattori favorenti i salti di specie.
Si pone altresì la necessità di un approccio multidisciplinare alle zoonosi emergenti nell'ambito della filosofia "One Health", coordinando specialisti di varie branche per creare reti e laboratori di sorveglianza attiva e pronto intervento, condividendo informazioni e database.

Un esempio è dato dal progetto PREDICT lanciato dall'agenzia statunitense per lo sviluppo internazionale, finalizzato al rilevamento precoce di potenziali zoonosi specialmente di origine virale, prima che diventino un problema per l'uomo.

Riferimento bibliografico

Kelly TR et al. Implementing One Health approaches to confront emerging and re-emerging zoonotic disease threats: lessons from PREDICT. One Health Outlook 2020; 2:1.

Segue articolo full text originale

Kelly et al. One Health Outlook (2020) 2:1
https://doi.org/10.1186/s42522-019-0007-9

One Health Outlook

REVIEW Open Access

Implementing One Health approaches to confront emerging and re-emerging zoonotic disease threats: lessons from PREDICT

Terra R. Kelly[1*], Catherine Machalaba[2], William B. Karesh[2], Paulina Zielinska Crook[1], Kirsten Gilardi[3], Julius Nziza[3], Marcela M. Uhart[1], Erika Alandia Robles[4], Karen Saylors[5], Damien O. Joly[5], Corina Monagin[1], Prime Mulembakani Mangombo[5], Placide Mbala Kingebeni[5], Rudovick Kazwala[6], David Wolking[1], Woutrina Smith[1], PREDICT Consortium and Jonna A. K. Mazet[1]

Abstract

Recurring outbreaks of emerging and re-emerging zoonoses, such as Ebola virus disease, avian influenza, and Nipah virus, serve as a reminder that the health of humans, animals, and the environment are interconnected and that early response to emerging zoonotic pathogens requires a coordinated, interdisciplinary, cross-sectoral approach. As our world becomes increasingly connected, emerging diseases pose a greater threat, requiring coordination at local, regional, and global levels. One Health is a multisectoral, transdisciplinary, and collaborative approach promoted to more effectively address these complex health threats. Despite strong advocacy for One Health, challenges for practical implementation remain. Here we discuss the value of the One Health approach for addressing global health challenges. We also share strategies applied to achieve successful outcomes through the USAID Emerging Pandemic Threats Program PREDICT project, which serve as useful case studies for implementing One Health approaches. Lastly, we explore methods for promoting more formal One Health implementation to capitalize on the added value of shared knowledge and leveraged resources.

Keywords: Emerging infectious diseases, Global health, One Health, Zoonotic diseases

Background

Zoonoses lead to millions of deaths annually; the economic losses from a single outbreak can amount to billions of dollars [1, 2]. Recurring outbreaks of emerging and re-emerging zoonotic infectious diseases, such as Ebola virus disease (EVD), severe acute respiratory syndrome (SARS), avian influenza (e.g. H5N1, H7N9), and Nipah virus disease underscore the need to consider the interconnections among the health of humans, animals, and the environment in disease prevention and control measures. As trade and travel facilitate greater access and connections across the world, these zoonoses pose significant and growing global health threats.

Lessons learned from these disease outbreaks highlight the need to shift to a more integrated, holistic, and proactive paradigm, such as can be achieved using the One Health approach. One Health considers the linkages among the health of humans, animals, plants, and their shared environment. As such, the approach allows for a deeper understanding and ability to address the complex eco-social determinants of health and to more effectively and efficiently tackle threats through coordination across disciplines and sectors. One Health approaches are increasingly recognized for their value in addressing emerging infectious disease (EID) threats, as the majority of EIDs arise from wild animal reservoirs in biodiverse

* Correspondence: trkelly@ucdavis.edu
[1]One Health Institute, University of California, Davis, CA, USA
Full list of author information is available at the end of the article

landscapes experiencing strong anthropogenic pressures, including human population growth, land use change, and natural resource extraction [3].

At a global level, there is broad support for the concept, which has led to the establishment of several One Health initiatives around the world, including designated divisions within U.S. federal agencies (e.g., in the U.S. the National Park Service One Health Initiative, Centers for Disease Control and Prevention One Health Office, and U.S. Department of Agriculture One Health Coordination Center), interagency working groups and national multisectoral coordination mechanisms (such as Bangladesh's One Health Secretariat and Liberia's One Health Coordination Platform) [4–6], international One Health networks and consortia (e.g., the FAO/OIE/WHO Tripartite collaboration, One Health Workforce, One Health Alliance of South Asia, Southeast Asia One Health University Network, One Health Central and Eastern Africa) and One Health designated degree and training programs [7–12]. Furthermore, nearly 50 countries have signed on to the Global Health Security Agenda (GHSA), which was launched in 2014 to bring countries together to promote One Health approaches and strengthen capacities to prevent, detect, and respond to disease threats [13].

Despite this broad support, implementing One Health approaches in practice still proves challenging. For instance, most countries lack formal mechanisms for coordination and integration of activities across the human health, agricultural, and environmental sectors, which are traditionally based in separate ministries or government agencies with different mandates on activities and spending [4, 14]. As a result, practical applications of One Health approaches have largely been ad-hoc [4, 15], resulting in delayed or incomplete prevention and control measures. There is also a need for formal standardized analyses showing the added benefits of One Health over conventional approaches in disease prevention and control [14, 16]. A growing body of research, including studies revealing the financial benefits of One Health investments in addressing emerging zoonoses, is building the evidence base for One Health [17, 18]. However, additional case studies and formal assessments demonstrating the social, health, and economic benefits are needed to garner broader high-level support by decision makers.

In 2009, the US Agency for International Development (USAID) launched the Emerging Pandemic Threats (EPT) Program's PREDICT Project. PREDICT utilizes a One Health approach focused on early detection and response to potentially zoonotic viral threats at their source ideally before they emerge in people [19]. PREDICT's efforts have focused on strengthening zoonotic virus surveillance and laboratory capacity in "hotspots" for EIDs. The project provided a platform for breaking down barriers through development of cross-sectoral surveillance and laboratory networks with open sharing of data, coordination on disease outbreak response, and contributions to extant or new national One Health platforms. PREDICT's efforts to operationalize One Health in collaboration with government and university partners provide valuable examples and evidence for the importance of One Health approaches in addressing complex health challenges. Here we discuss the value of One Health for addressing complex health threats at the human-animal-environment interface and current hurdles for implementing One Health. We also share approaches used by PREDICT to achieve successful outcomes, which serve as useful case studies for applying the One Health approach.

Value of the One Health approach

The One Health approach builds on existing capacities but is novel in bringing disciplines and sectors together to provide broader health benefits. Increasing cross-sectoral coordination can help promote science-based decision making; reduce unnecessary duplication among the sectors responsible for the health of humans, animals, and the environment; and more effectively address outside factors influencing disease burdens [2, 18].

Comparative medicine has long been acknowledged for its benefits in scientific research, and One Health expands comparative medicine's scope to surveillance in animals and the environment for early detection and better understanding of threats to mitigate risk and impacts. For example, great ape die-offs associated with Ebola virus have often been detected prior to outbreaks in humans, providing a potential predictive value that can help prevent human cases if paired with risk mitigation measures, such as hunter avoidance of carcasses [20]. Weather conditions have also been used to forecast Rift Valley fever and other outbreaks and can inform vaccination and mosquito control campaigns to reduce health and economic consequences of disease epidemics [21]. Integrated human, animal, and environmental surveillance can likewise elucidate pathways of pathogen sharing and inform development of more comprehensive solutions that emphasize prevention at the source.

The onset of encephalitis cases in people and birds that were ultimately linked to the emergence of West Nile virus in the U.S. in 1999 left public health authorities challenged with identifying its origin. Critical insight into the cause of disease was gained from the veterinary community investigating associated wild bird mortalities. Currently, sentinel surveillance in mosquitos, birds, and horses is used routinely to monitor risk to human health and trigger preventive measures. Parts of North America and Western Europe have also made a

concerted effort to control rabies using a One Health approach. While effective rabies control efforts have required substantial investments, they have yielded high public health benefits, with canine vaccination widely considered the most cost-effective strategy [22–24]. The successful control of rabies in dogs through vaccination has then allowed for a targeted approach to managing wildlife reservoirs. Baseline surveillance data has enabled managers to monitor risk and target control efforts in these populations, as seen in response to the rise of raccoon rabies.

An economic optimization projection suggested that investing in a One Health approach through mitigation of pandemic threats versus business-as-usual adaptation could yield a savings of over $300 billion globally over the next century [17]. Similarly, a World Bank analysis suggested that upfront investments of $3.4 billion per year globally in One Health capacity through improved veterinary and public health services could avoid over $30 billion in zoonotic disease response annually worldwide [2].

While these scenarios reflect value for global public good, countries are also increasingly endorsing health security as a national priority given the potential for rapid disease spread via trade and travel networks. This necessitates improved prevention and control of both endemic and emerging disease risks within and beyond a nation's borders. Climate and other ecological changes are resulting in shifts in geographic ranges of species and their pathogens with a wide range of associated ongoing and novel health threats – ranging from vector-borne and zoonotic diseases to impacts on food safety and security. For example, the spread of Zika virus and CDC's request to the U.S. government for $1.8 billion to respond demonstrate the need for One Health approaches to implement preventive measures prior to the emergence of novel health threats.

Case studies: One Health contributions toward more efficient and effective response to emerging zoonotic disease threats

Over the past decade, PREDICT partnered with foreign governments, universities, and other organizations to advance One Health initiatives [19]. In collaboration with local partners, the PREDICT project strengthened capacity for viral surveillance at high-risk animal-human interfaces. Also, when requested by host country government partners, PREDICT provided support during disease outbreaks by incorporating animal sampling into investigations, expanding laboratory analyses to look for novel viruses, and promoting the growth of a trained One Health workforce.

Rapid outbreak response and containment

During the widespread EVD outbreak in West Africa in 2014, the Democratic Republic of Congo (DRC) experienced its own separate and unique Ebola virus disease outbreak. Unlike West Africa, DRC has a long history of Ebola outbreaks and substantial capacity for response, due in part to a long-running partnership between l'Institut National de Recherche Biomédicale (INRB), the national infectious disease reference laboratory, and other partners like PREDICT. Many experts from the Viral Hemorrhagic Fever Unit of the INRB were deployed in West Africa when the outbreak in DRC occurred. As a result, PREDICT was requested to support laboratory testing. Suspect cases were sampled, specimens were shipped to the PREDICT laboratory at INRB for analyses, and Ebola virus was detected within 1 day of receiving the specimens. Importantly, the strain of Ebola virus detected was distinct from the strain causing the West Africa epidemic, ruling out linkages between the two outbreaks. Following the prompt testing and pathogen identification, the DRC government was able to access the affected area and respond rapidly with contact tracing, dispatching a mobile laboratory, and quarantining suspected cases, leading to swift containment with only 66 cases reported over the two-month duration of this outbreak.

The PREDICT team was also able to assist with collection of wildlife samples from the outbreak area. Contact tracing later identified the likely source of the outbreak as an infected wild animal that had been found dead and butchered for food. This information was key to identifying high-risk practices to target for disease prevention. The rapid response and field investigations informing on prevention measures illustrate what is achievable when an in-country One Health workforce is trained, employed, and ready to act. Such prevention arguably becomes even more important when country capacity to rapidly respond to outbreaks is lacking, especially in fragile areas of high vulnerability to both disease threats and their impacts (e.g. resulting from weak governance structures). The impacts of the on-going EVD outbreak in DRC, which began in Kivu DRC in August 2018, highlight the challenge of responding to a disease outbreak in a remote location where access and control efforts have been substantially impeded by violence and insurgency. These reinforce the need for continued capacity strengthening and integration of sectors at national and sub-national levels, tailored to the local risk context and stakeholders to promote relevance, sustainability, and ownership.

Prevention of human disease outbreaks

Currently, response to outbreaks around the world is highly reactive, with control measures employed once an

outbreak in humans has been detected. PREDICT activities in Bolivia demonstrated that monitoring for zoonotic viruses in wild animals can be a valuable early detection tool for preventing disease outbreaks, particularly in landscapes undergoing substantial alteration, such as deforestation, where breakdown of natural barriers leads to increased contact between wildlife and people.

Yellow fever (YF) is a zoonotic viral hemorrhagic disease [25] that is perpetuated in a transmission cycle involving mosquitos and non-human primate hosts. Because New World primate hosts are especially susceptible to YFV infection, acute clusters of mortality in these populations can signal YFV activity and alert authorities to increased risk of human infection, thereby serving as an early warning system.

In 2012, staff at a wildlife sanctuary in Bolivia, who had received training in wildlife disease surveillance through PREDICT, discovered six dead howler monkeys (*Alouatta sara*) near the park. In collaboration with the sanctuary, PREDICT investigated the mortality event. Post-mortem examinations and diagnostic testing performed at the University of San Andres' Institute of Molecular Biology and Biotechnology, PREDICT's partner laboratory in Bolivia, indicated infection by a flavivirus, the family of viruses to which YFV belongs. PREDICT partners reported the results to the Ministry of Health, while conducting further laboratory analyses to confirm that infection was caused by YFV. The Ministry of Health, Pan-American Health Organization, and PREDICT conducted a joint risk assessment followed by a prompt cross-sectoral, coordinated response in the affected area. The response included preventive YF human vaccination, public education and outreach, and mosquito control to reduce risk of infection.

Although YF outbreaks had never been documented in Bolivian primates, authorities were able to implement preventive measures in the surrounding area within 1 week of detection of the mortality event. No human cases of YF were subsequently reported, suggesting the value of early warning systems for increased zoonotic disease risk, local pathogen detection capacity, effective collaboration channels across sectors, and prompt implementation of public health measures for preventing pathogen spillover from animals into people.

Systematic coordinated data sharing and national One Health platforms

PREDICT worked with foreign government partners to establish a systematic One Health approach to communicating findings stemming from disease surveillance. The process involved sharing laboratory results with designated points of contact in the ministries representing public health, livestock/agriculture, and wildlife, which facilitated discussions on coordinated solutions. It also established open communication channels that enabled more rapid coordinated responses to disease outbreaks. In Rwanda and Tanzania, this collaborative approach was the impetus for PREDICT's involvement in the development of national One Health platforms in the countries.

In Rwanda, PREDICT-trained personnel served on the Government of Rwanda's One Health Steering Committee. The committee, which is made up by representatives from the animal and human health and environmental sectors, applied "a participatory and consensus building process" to develop an integrative framework for solving problems at the animal-human-environmental interface [26]. As part of the committee, PREDICT team members aided in the development of a One Health Strategic Plan in 2015 [26]. The plan references commitments to enhance cross-sectoral collaboration and increase One Health workforce capacity in Rwanda. It outlines an implementation strategy covering organizational structure and pooling and mobilizing resources [27]. The Steering Committee oversees the plan, including prioritization of resource allocations, and coordinates the technical aspects of the strategy, which are integrated into the annual action plans of the implementing partners. If successfully operationalized, Rwanda's One Health Strategic Plan will lead to more efficient and timely responses to disease threats [27].

For example, following the avian influenza (AI) outbreak in neighboring Uganda in 2017, the Rwanda Agriculture Board, in collaboration with representatives from the National One Health Steering Committee, conducted a field investigation of an avian mortality event in Rwanda. In the process of their investigation, they conducted public sensitization around AI risk through informal community meetings and radio broadcast. Although AI was not confirmed in Rwanda, the collaborative efforts initiated by the committee raised critical awareness and led to improvements in Rwanda's National Contingency Plan against AI highlighting the benefits of this plan to improving preparedness.

Alongside Rwanda, Tanzania also launched its One Health Strategic Plan in 2015. This plan laid the groundwork for multi-sectoral coordination and established a One Health Coordination Unit overseen by a One Health Steering Committee, comprised of secretaries of participating ministries and supported by five technical working groups. Tanzania was the first country to undergo a self-assessment using the World Health Organization (WHO) Joint External Evaluation (JEE) tool, which is a voluntary, collaborative process to assess a country's capacity to prevent, detect, and rapidly respond to public health threats [28]. PREDICT representatives served in one of the technical working groups

using the tool to evaluate strengths, gaps, and priority actions for enhancing national health security. The assessment was instrumental for encouraging cross-sectoral communication and identifying activities in which ministry partners could work together to combat disease threats. The process paved the way for developing the Tanzania National Action Plan for Health Security, which addresses gaps identified by the evaluation. As a culmination of these efforts, Tanzania formally launched the first national One Health Platform and One Health Strategic Plan in 2018 [29].

The way forward: implementing One Health

While mechanisms for operationalizing One Health are variable across contexts, case studies demonstrating successful One Health outcomes can provide valuable insight for implementing approaches elsewhere. These can be leveraged as countries work toward multisectoral coordination platforms with more sustainable approaches to One Health (such as through the establishment of the Zoonotic Disease Unit in Kenya [30]). These platforms often have high political will, with oversight and support at prime minister or presidential levels which promote country ownership and sustained attention and across sectors. Over the past 5 years, the GHSA has been instrumental in creating an enabling environment and political will for strengthening global and national health securities through a One Health approach. JEEs conducted in several countries around the world have revealed weaknesses in coordination across health sectors prompting the recommendation to develop national One Health platforms. To work towards this goal, the World Bank, USAID EPT program, and United Nations organization partners have compiled resources to assist countries with formalizing a One Health strategy, including tools for capacity assessments, resource mapping and prioritization, and One Health systems improvement [24, 31–37]. These tools aid in identifying where investments in One Health approaches and leveraged resources could fill gaps, avoid unnecessary overlap, and result in more holistic, preventive approaches [18]. In allocating resources, it is beneficial to conduct formal standardized assessments to evaluate how best to optimize investments to ensure added value gained by integrating efforts across health sectors [32, 33]. For example, One Health approaches have yielded higher returns on investments through joint human-animal disease surveillance and prevention and control measures, including vaccination campaigns [18, 19, 34]. Cross-sectoral exercises to assess risk and economic impacts of zoonoses have also brought stakeholders to the table to facilitate more systematic collaboration and communication and to identify opportunities of mutual benefit [18, 35, 36]. Leveraging the One Health approach to ensure the wider risk context and relevant sectors, especially at sub-national levels, can help boost countries' abilities to prepare for a suite of current and evolving threats.

Finally, it is critical to continue to raise awareness of One Health and foster leaders who are uniquely skilled to work across disciplines and sectors. Around the world, universities are progressively incorporating One Health education into their curricula, including designated degree programs. These programs need to be developed around a set of core competencies with an emphasis on practical skill-building [37] to provide students with the knowledge and experience necessary to address complex health threats.

Conclusions

While there is increasing commitment to One Health across the world, implementing One Health approaches in practice still proves challenging. Development of national One Health platforms and policies are critical for improving coordination and integration of activities and programs across sectors. In many countries, the GHSA has provided a platform for coordination and served as the impetus to initiate One Health strategic plans and to develop national One Health policies. In addition, support from international organizations, such as the World Bank, USAID (EPT Program), and UN partners has aided several countries in designing and implementing One Health strategies and in strengthening national One Health systems [18, 19]. While some programmatic activities may not be feasible in the absence of external funding, one route for sustainability is the application of low-cost coordination systems that have been tested and validated, including routine inter-ministry meetings to share disease surveillance results and discuss coordinated mitigation efforts. Country investments in human and animal health systems, including through development loans, illustrate the value that countries place on enhancing capacity for disease preparedness. Further, there is a need to continue to bring attention to the value of One Health approaches and to invest in training a workforce of One Health leaders who have the skills to think critically and work collaboratively across sectors.

Abbreviations

AI: Avian Influenza; CDC: Centers for Disease Control and Prevention; DRC: Democratic Republic of Congo; EID: Emerging Infectious Disease; EPT: Emerging Pandemic Threats; EVD: Ebola Virus Disease; GHSA: Global Health Security Agenda; INRB: l'Institut National de Recherche Biomédicale; JEE: Joint External Evaluation; UN: United Nations; USAID: US Agency for International Development; WHO: World Health Organization; YF: Yellow Fever

Acknowledgements

This work was made possible by the generous support of the American people through the USAID. The contents are the responsibility of the PREDICT authors and do not necessarily reflect the views of USAID or the U.S. Government. We thank the governments and partners in PREDICT countries.

Authors' contributions
TRK, CM, WBK, PZC, and JM conceived the study and were major contributors in writing the manuscript. KG and JN led the PREDICT project's activities in Rwanda and contributed to the writing of the manuscript. MMU and EAR led the PREDICT project's activities in Bolivia and contributed to the writing of the manuscript. KS, DOJ, CM, PMM, and PMK led the PREDICT project's activities in the DRC and contributed to the writing of the manuscript. RK, DW, JM, and WS led the project's activities in Tanzania and contributed to the writing of the manuscript. The PREDICT Consortium is a consortium of researchers who have made substantial contributions to the design and implementation of the PREDICT project. All authors read and approved the final manuscript.

Funding
This work was made possible by the generous support of the American people through the USAID. The USAID did not play a role in conceiving or writing this manuscript.

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]One Health Institute, University of California, Davis, CA, USA. [2]EcoHealth Alliance, New York, NY, USA. [3]Gorilla Doctors, Mountain Gorilla Veterinary Project and Karen C. Drayer Wildlife Health Center, University of California, Davis, CA, USA. [4]Bolivian Network of Primatology, La Paz, Bolivia. [5]Metabiota, San Francisco, CA, USA. [6]Sokoine University of Agriculture, Morogoro, Tanzania.

Received: 24 May 2019 Accepted: 13 December 2019
Published online: 10 January 2020

References

1. Lee J, McKibbin WJ. Globalization and disease: the case of SARS. In: Brooking Institution, ed. Brookings Discussion Papers in International Economics; 2004.
2. The World Bank. People, Pathogens, and Our Planet: The Economics of One Health, vol. 2; 2012. Report #69145-GLB
3. Karesh WB, Dobson A, Lloyd-Smith JO, et al. Ecology of zoonoses: natural and unnatural histories. Lancet. 2012;380:1936–45. https://doi.org/10.1016/S0140-6736(12)61678-X.
4. Rubin C, Dunham B, Sleeman J. Making One Health a reality - Crossing bureaucratic boundaries. Microbiol Spectr. 2014;2(1):OH-0016–2012. https://doi.org/10.1128/microbiolspec OH-0016-2012.
5. Machalaba CC, Salerno RH, Barton Behravesh C, et al. Institutionalizing One Health: From assessment to action. Heal Secur. 2018;16(S1):S-37–43. https://doi.org/10.1089/hs.2018.0064.
6. Government of Liberia. One Health Governance Manual; 2018.
7. Arizona State University. MPH One Health. 2019. https://publichealth.arizona.edu/academics/masters/mph/one-health. Accessed 14 Aug 2019.
8. University of Florida. MHS in One Health. 2019. https://egh.phhp.ufl.edu/prospective-students-2/degree-programs/mhs-one-health/. Accessed 14 Aug 2019.
9. University of California. Rx One Health. 2019. www.vetmed.ucdavis.edu/ohi/programs/rx-one-health.cfm. Accessed 14 Aug 2019.
10. EcoHealth Alliance. EcoHealth net. 2019. https://www.ecohealthalliance.org/program/ecohealthnet. Accessed 15 Aug 2019.
11. Ruiz de Castaneda R, Garrison A, Haeberli P, et al. First "Global Flipped Classroom in One Health". From MOOCs to research on real world challenges. One Heal. 2018;5:37–9. https://doi.org/10.1016/j.onehlt.2018.02.001.
12. Uehlinger FD, Freeman DA, Waldner CL. The One Health leadership experience at the University of Saskatchewan, Canada. J Vet Med Educ. 2019;46(2):172–83.
13. Global Health Security Agenda. Global Health Security Agenda. 2019. https://www.ghsagenda.org/about. Accessed 14 Aug 2019.
14. Lee K, Brumme ZL. Operationalizing the One Health approach: the global governance challenges. Health Policy Plan. 2012;28(7):778–85. https://doi.org/10.1093/heapol/czs127 .
15. Gibbs EP. The evolution of One Health: a decade of progress and challenges for the future. Vet Rec. 2014;174(4):85–91.
16. Rushton J, Nielsen LR, Cornelsen L, Qeenan K, et al. In: Ruegg SR, Hasler B, Zinsstag J, editors. Evaluation of integrated approaches to health with a focus on One Health. In: Integrated Approaches to Health: A Handbook for the Evaluation of One Health. Wageningen, Netherlands: Wageningen Academic Publishers; 2018.
17. Pike J, Bogich T, Elwood S, Finnoff D, Daszak P. Economic optimization of a global strategy to address the pandemic threat. Proc Natl Acad Sci U S A. 2014;111(52):18519–23.
18. FCJ B, Bouley T, Karesh WB, Le Gall FG, Machalaba CC, Plante CA, Seifman RM. Operational framework for strengthening human, animal and environmental public health systems at their interface (English). Washington, DC: World Bank Group; 2018. http://documents.worldbank.org/curated/en/703711517234402168/Operational-framework-for-strengthening-human-animal-and-environmental-public-health-systems-at-their-interface http://documents.worldbank.org/curated/en/703711517234402168/pdf/123023-REVISED-PUBLIC-World-Bank-One-Health-Framework-2018.pdf
19. PREDICT Consortium. Reducing Pandemic Risk, Promoting Global Health. 2014. Davis, CA. https://ohi.sf.ucdavis.edu/sites/g/files/dgvnsk5251/files/files/page/predict-final-report-lo.pdf .
20. Rouquet P, Froment JM, Bermejo M, et al. Wild animal mortality monitoring and human ebola outbreaks, Gabon and Republic of Congo, 2001-2003. Emerg Infect Dis. 2005;11(2):283–90. https://doi.org/10.3201/eid1102.040533 .
21. Anyamba A, Chretien JP, Small J, et al. Prediction of a Rift Valley fever outbreak. Proc Natl Acad Sci U S A. 2009;106:955–9.
22. Centers for Disease Control and Prevention. Rabies. 2019. https://www.cdc.gov/rabies/. Accessed 26 Aug 2019.
23. World Organisation for Animal Health (OIE). Dog vaccination: the key to end dog-transmitted human rabies. 2018. https://www.oie.int/en/for-the-media/press-releases/detail/article/dog-vaccination-the-key-to-end-dog-transmitted-human-rabies/. Accessed 26 Aug 2019.
24. Fitzpatrick MC, Shah HA, Pandey A, et al. One Health approach to cost-effective rabies control in India. Proc Natl Acad Sci. 2016;113(51):14574. https://doi.org/10.1073/pnas.1604975113 .
25. Monath TP. Yellow fever: an update. Lancet Infect Dis. 2001;1(1):11–20.
26. Ministry of Health Republic of Rwanda. One Health Strategic Plan. 2015. http://www.rbc.gov.rw/IMG/pdf/one_health.pdf.
27. Nyatanyi T, Wilkes M, McDermott H, et al. Implementing One Health as an integrated approach to health in Rwanda. BMJ Glob Health. 2017;2(1):e000121. https://doi.org/10.1136/bmjgh-2016-000121 .
28. Global Health Security Agenda. Assessments and JEE. 2018. https://www.ghsagenda.org/assessments. Accessed 24 May 2018.
29. Tanzania Prime Ministry Launches One Health Platform and Strategic Plan. Preparedness and Response. 2018.
30. Falzon LC, Alumasa L, Amanya F, Kang'he E, Kariuki S, Momanyi K, Muinde P, Murungi MK, Njoroge SM, Ogendo A, Ogola J, Rushton J, Woolhouse MEJ, Fevre EM. One Health in action: Operational aspects of an integrated surveillance system for zoonoses in western Kenya. Front Vet Sci. 2019;6:252. https://doi.org/10.3389/fvets.2019.00252.
31. One Health Commission, Smith T, Young T. Advancing Emergency Preparedness Through One Health Act of 2018. 2018;189(18):A.2615 http://www.onehealthinitiative.com/publications/S.2615OnePager-Call to Action.pdf.
32. Baum SE, Machalaba C, Daszak P, Salerno RH, Karesh WB. Evaluating One Health: are we demonstrating effectiveness? One Heal. 2017;3:5–10. https://doi.org/10.1016/j.onehlt.2016.10.004 .
33. Schar DL, Yamey GM, Machalaba CC, Karesh W. A framework for stimulating economic investments to prevent emerging diseases. Bull WHO. 2018;96:138–40.
34. Schelling E, Bechir M, Ahmed MA, Wyss K, Randolph TF, Zinsstag J. Human and animal vaccination delivery to remote nomadic families, Chad. Emerg Infect Dis. 2007;13(3):373–9.

35. American Public Health Association. Advancing a "One Health" approach to promote health at the human-animal-environment interface. 2017. https://www.apha.org/policies-and-advocacy/public-health-policy-statements/policy-database/2018/01/18/advancing-a-one-health-approach . Accessed 26 Aug 2019.
36. Machalaba C, Smith KM, Awada L, et al. One Health economics to confront disease threats. Trans R Soc Trop Med Hyg. 2017;111(6):235–7.
37. Togami E, Gardy JL, Columbia B, et al. Core competencies in One Health education: What are we missing? NAM Perspect. Washington, DC: Discussion paper, National Academy of Medicine; 2018. https://doi.org/10.31478/201806a .

Publisher's Note

COVID-19: scenari terapeutici in divenire

A circa due mesi dal riconoscimento dell'epidemia di COVID-19 ancora non è disponibile una strategia terapeutica universalmente riconosciuta e validata.
Ciò è in parte un retaggio della mancanza di evidenze conclusive relativamente alle sindromi (SARS e MERS) causate dai precedenti coronavirus emergenti di questo secolo.
In aggiunta, in poche settimane è difficile ottenere dati solidi dai numerosi trial clinici che pure sono stati organizzati con grande velocità, specialmente in Cina dove è concentrata la maggioranza dei pazienti. Specialmente per i casi più lievi può essere difficile stabilire se un intervento terapeutico modifica significativamente la prognosi, probabilmente intrinsecamente già benigna. Diverso è il discorso per le forme più gravi, nelle quali interventi terapeutici efficaci sono attesi.
Allo stato attuale si stanno testando sia farmaci "vecchi", riproposti ("repurposed") sulla base di dati in vitro, che "nuovi": al primo gruppo appartiene per esempio l'antimalarico clorochina, al secondo l'analogo nucleotidico remdesivir, testato nel recento passato per Ebol.a.

Riferimento bibliografico

Wang M et al. Cell Research, febbraio 2020 (letter to the editor) Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020; 30: 269-271.

Segue articolo full text originale

Cell Research

www.nature.com/cr
www.cell-research.com

LETTER TO THE EDITOR OPEN

Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro

Cell Research (2020) 0:1–3; https://doi.org/10.1038/s41422-020-0282-0

Dear Editor,

In December 2019, a novel pneumonia caused by a previously unknown pathogen emerged in Wuhan, a city of 11 million people in central China. The initial cases were linked to exposures in a seafood market in Wuhan.[1] As of January 27, 2020, the Chinese authorities reported 2835 confirmed cases in mainland China, including 81 deaths. Additionally, 19 confirmed cases were identified in Hong Kong, Macao and Taiwan, and 39 imported cases were identified in Thailand, Japan, South Korea, United States, Vietnam, Singapore, Nepal, France, Australia and Canada. The pathogen was soon identified as a novel coronavirus (2019-nCoV), which is closely related to sever acute respiratory syndrome CoV (SARS-CoV).[2] Currently, there is no specific treatment against the new virus. Therefore, identifying effective antiviral agents to combat the disease is urgently needed.

An efficient approach to drug discovery is to test whether the existing antiviral drugs are effective in treating related viral infections. The 2019-nCoV belongs to *Betacoronavirus* which also contains SARS-CoV and Middle East respiratory syndrome CoV (MERS-CoV). Several drugs, such as ribavirin, interferon, lopinavir-ritonavir, corticosteroids, have been used in patients with SARS or MERS, although the efficacy of some drugs remains controversial.[3] In this study, we evaluated the antiviral efficiency of five FAD-approved drugs including ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine and two well-known broad-spectrum antiviral drugs remdesivir (GS-5734) and favipiravir (T-705) against a clinical isolate of 2019-nCoV in vitro.

Standard assays were carried out to measure the effects of these compounds on the cytotoxicity, virus yield and infection rates of 2019-nCoVs. Firstly, the cytotoxicity of the candidate compounds in Vero E6 cells (ATCC-1586) was determined by the CCK8 assay. Then, Vero E6 cells were infected with nCoV-2019BetaCoV/Wuhan/WIV04/2019[2] at a multiplicity of infection (MOI) of 0.05 in the presence of varying concentrations of the test drugs. DMSO was used in the controls. Efficacies were evaluated by quantification of viral copy numbers in the cell supernatant via quantitative real-time RT-PCR (qRT-PCR) and confirmed with visualization of virus nucleoprotein (NP) expression through immunofluorescence microscopy at 48 h post infection (p.i.) (cytopathic effect was not obvious at this time point of infection). Among the seven tested drugs, high concentrations of three nucleoside analogs including ribavirin (half-maximal effective concentration (EC_{50}) = 109.50 μM, half-cytotoxic concentration (CC_{50}) > 400 μM, selectivity index (SI) > 3.65), penciclovir (EC_{50} = 95.96 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.88 μM, CC_{50} > 400 μM, SI > 6.46) were required to reduce the viral infection (Fig. 1a and Supplementary information, Fig. S1). However, favipiravir has been shown to be 100% effective in protecting mice against Ebola virus challenge, although its EC_{50} value in Vero E6 cells was as high as 67 μM,[4] suggesting further in vivo studies are recommended to evaluate this antiviral nucleoside. Nafamostat, a potent inhibitor of MERS-CoV, which prevents membrane fusion, was inhibitive against the 2019-nCoV infection (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44). Nitazoxanide, a commercial antiprotozoal agent with an antiviral potential against a broad range of viruses including human and animal coronaviruses, inhibited the 2019-nCoV at a low-micromolar concentration (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76). Further in vivo evaluation of this drug against 2019-nCoV infection is recommended. Notably, two compounds remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) and chloroquine (EC_{50} = 1.13 μM; CC_{50} > 100 μM, SI > 88.50) potently blocked virus infection at low-micromolar concentration and showed high SI (Fig. 1a, b).

Remdesivir has been recently recognized as a promising antiviral drug against a wide array of RNA viruses (including SARS/MERS-CoV[5]) infection in cultured cells, mice and nonhuman primate (NHP) models. It is currently under clinical development for the treatment of Ebola virus infection.[6] Remdesivir is an adenosine analogue, which incorporates into nascent viral RNA chains and results in pre-mature termination.[7] Our time-of-addition assay showed remdesivir functioned at a stage post virus entry (Fig. 1c, d), which is in agreement with its putative antiviral mechanism as a nucleotide analogue. Warren et al. showed that in NHP model, intravenous administration of 10 mg/kg dose of remdesivir resulted in concomitant persistent levels of its active form in the blood (10 μM) and conferred 100% protection against Ebola virus infection.[7] Our data showed that EC_{90} value of remdesivir against 2019-nCoV in Vero E6 cells was 1.76 μM, suggesting its working concentration is likely to be achieved in NHP. Our preliminary data (Supplementary information, Fig. S2) showed that remdesivir also inhibited virus infection efficiently in a human cell line (human liver cancer Huh-7 cells), which is sensitive to 2019-nCoV.[2]

Chloroquine, a widely-used anti-malarial and autoimmune disease drug, has recently been reported as a potential broad-spectrum antiviral drug.[8,9] Chloroquine is known to block virus infection by increasing endosomal pH required for virus/cell fusion, as well as interfering with the glycosylation of cellular receptors of SARS-CoV.[10] Our time-of-addition assay demonstrated that chloroquine functioned at both entry, and at post-entry stages of the 2019-nCoV infection in Vero E6 cells (Fig. 1c, d). Besides its antiviral activity, chloroquine has an immune-modulating activity, which may synergistically enhance its antiviral effect in vivo. Chloroquine is widely distributed in the whole body, including lung, after oral administration. The EC_{90} value of chloroquine against the 2019-nCoV in Vero

Received: 25 January 2020 Accepted: 28 January 2020
Published online: 04 February 2020

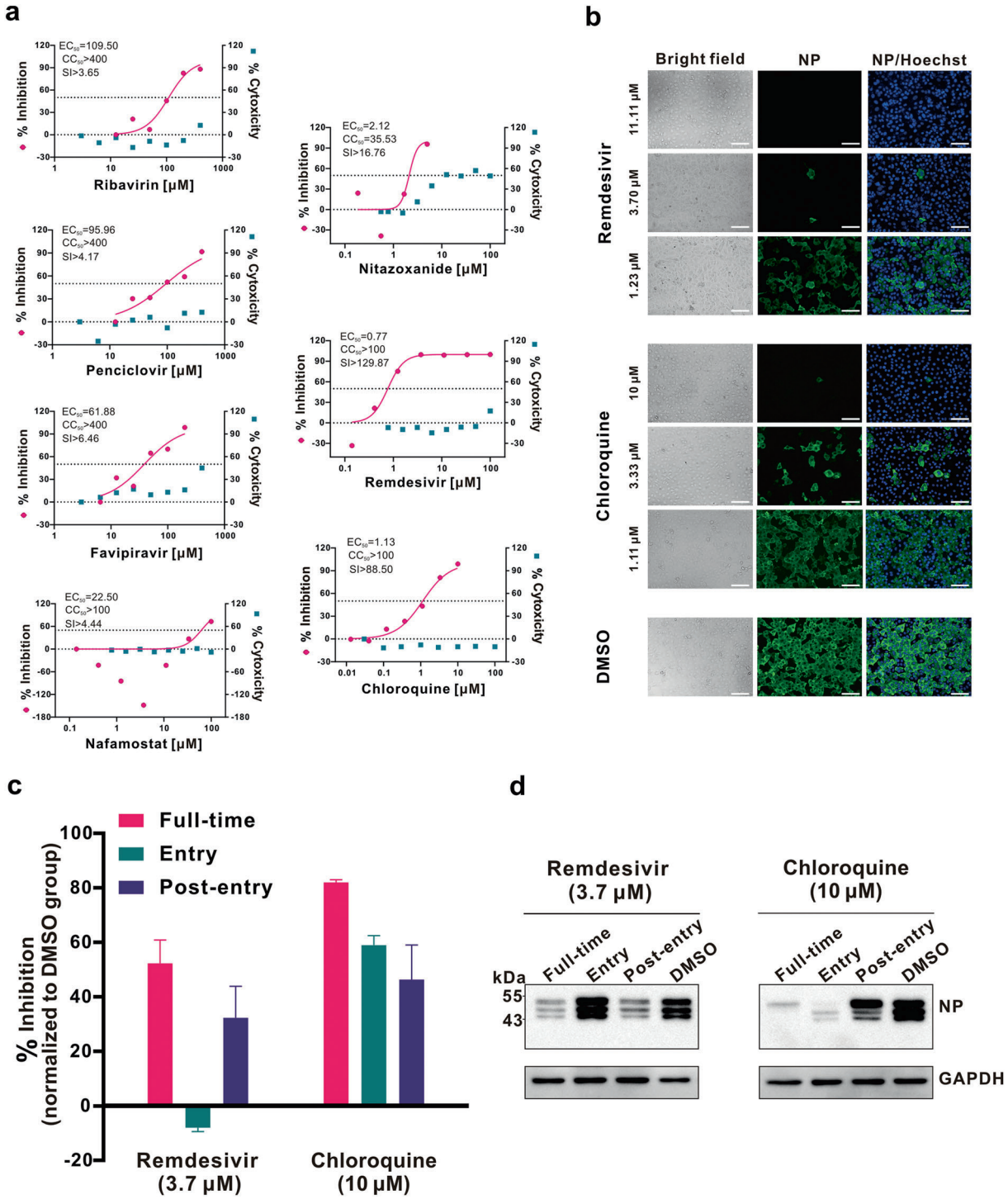


Fig. 1 The antiviral activities of the test drugs against 2019-nCoV in vitro. a Vero E6 cells were infected with 2019-nCoV at an MOI of 0.05 in the treatment of different doses of the indicated antivirals for 48 h. The viral yield in the cell supernatant was then quantified by qRT-PCR. Cytotoxicity of these drugs to Vero E6 cells was measured by CCK-8 assays. The left and right *Y*-axis of the graphs represent mean % inhibition of virus yield and cytotoxicity of the drugs, respectively. The experiments were done in triplicates. **b** Immunofluorescence microscopy of virus infection upon treatment of remdesivir and chloroquine. Virus infection and drug treatment were performed as mentioned above. At 48 h p.i., the infected cells were fixed, and then probed with rabbit sera against the NP of a bat SARS-related CoV[2] as the primary antibody and Alexa 488-labeled goat anti-rabbit IgG (1:500; Abcam) as the secondary antibody, respectively. The nuclei were stained with Hoechst dye. Bars, 100 μm. **c** and **d** Time-of-addition experiment of remdesivir and chloroquine. For "Full-time" treatment, Vero E6 cells were pre-treated with the drugs for 1 h, and virus was then added to allow attachment for 2 h. Afterwards, the virus–drug mixture was removed, and the cells were cultured with drug-containing medium until the end of the experiment. For "Entry" treatment, the drugs were added to the cells for 1 h before viral attachment, and at 2 h p.i., the virus–drug mixture was replaced with fresh culture medium and maintained till the end of the experiment. For "Post-entry" experiment, drugs were added at 2 h p.i., and maintained until the end of the experiment. For all the experimental groups, cells were infected with 2019-nCoV at an MOI of 0.05, and virus yield in the infected cell supernatants was quantified by qRT-PCR **c** and NP expression in infected cells was analyzed by Western blot **d** at 14 h p.i.

E6 cells was 6.90 μM, which can be clinically achievable as demonstrated in the plasma of rheumatoid arthritis patients who received 500 mg administration.[11] Chloroquine is a cheap and a safe drug that has been used for more than 70 years and, therefore, it is potentially clinically applicable against the 2019-nCoV.

Our findings reveal that remdesivir and chloroquine are highly effective in the control of 2019-nCoV infection in vitro. Since these compounds have been used in human patients with a safety track record and shown to be effective against various ailments, we suggest that they should be assessed in human patients suffering from the novel coronavirus disease.

ACKNOWLEDGEMENTS

We thank Xi Wang, Yan Wu, Weijuan Shang, Huanyu Zhang, Yufeng Li, Hengrui Hu, Xiaming Jiang, Yuan Sun, from Wuhan Institute of Virology for their essential assistance with this study. We thank Prof. Fei Deng from National Virus Resource Center, and Tao Du, Jia Wu and Hao Tang from BSL-3 Laboratory of Wuhan Institute of Virology for their critical support. We thank Prof. Yanyi Wang and other colleagues of Wuhan Institute of Virology and Wuhan National Biosafety Laboratory for their excellent coordination. We thank Dr. Basil Arif for scientific editing of the manuscript. We thank the anonymous reviewers for their valuable suggestions. This work was supported in part by grants from the National Science and Technology Major Projects for "Major New Drugs Innovation and Development" (directed by Prof. Song Li) (2018ZX09711003), the National Natural Science Foundation of China (31621061), and the Emergency Scientific Research Project for 2019-nCoV from Hubei Province (to Profs. Zhengli Shi and Gengfu Xiao).

AUTHOR CONTRIBUTIONS

G.X., W.Z., Z.H., M.W., R.C., and L.Z. conceived and designed the experiments. X.Y., J.L., M.X., M.W., R.C., and L.Z. participated in multiple experiments; G.X., W.Z., Z.H., Z.S., M.W., R.C., and L.Z. analyzed the data. M.W., L.Z., R.C., and Z.H. wrote the manuscript. G.X., W.Z., and Z.H. provided the final approval of the manuscript.

ADDITIONAL INFORMATION

Supplementary information accompanies this paper at https://doi.org/10.1038/s41422-020-0282-0.

Competing interests: The authors declare no competing interests.

Manli Wang[1], Ruiyuan Cao[2], Leike Zhang[1], Xinglou Yang[1], Jia Liu[1], Mingyue Xu[1], Zhengli Shi[1], Zhihong Hu[1], Wu Zhong[2] and Gengfu Xiao[1]

[1]State Key Laboratory of Virology, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, 430071 Wuhan, China and [2]National Engineering Research Center for the Emergency Drug, Beijing Institute of Pharmacology and Toxicology, 100850 Beijing, China

These authors contributed equally: Manli Wang, Ruiyuan Cao, Leike Zhang, Xinglou Yang.

Correspondence: Zhihong Hu (huzh@wh.iov.cn) or Wu Zhong (zhongwu@bmi.ac.cn) or Gengfu Xiao (xiaogf@wh.iov.cn)

REFERENCES

1. Huang, C. L. et al. *The Lancet* https://doi.org/10.1016/S0140-6736(20)30183-5 (2020).
2. Zhou, P. et al. Nature (accepted).
3. Zumla, A., Chan, J. F., Azhar, E. I., Hui, D. S. & Yuen, K. Y. *Nat. Rev. Drug Discov.* **15**, 327–347 (2016).
4. Oestereich, L. et al. *Antivir. Res.* **105**, 17–21 (2014).
5. Sheahan, T. P. et al. *Sci. Transl. Med.* **9**, eaal3653 (2017).
6. Mulangu, S. et al. *N. Engl. J. Med.* **381**, 2293–2303 (2019).
7. Warren, T. K. et al. *Nature* **531**, 381–385 (2016).
8. Savarino, A., Di Trani, L., Donatelli, I., Cauda, R. & Cassone, A. *Lancet Infect. Dis.* **6**, 67–69 (2006).
9. Yan, Y. et al. *Cell Res.* **23**, 300–302 (2013).
10. Vincent, M. J. et al. *Virol. J.* **2**, 69 (2005).
11. Mackenzie, A. H. *Am. J. Med.* **75**, 40–45 (1983).

L'epidemia da SARS-COV-2 ai tempi dei social network: non solo infodemia ma anche preziosa fonte di informazioni

L'emergenza globale legata a COVID-19 rappresenta la prima epidemia ai tempi dei social network. Ciò è stato correlato al deleterio fenomeno dell'infodemia, ovvero un eccesso non regolato di informazioni spesso non vagliate e inattendibili, che rendono difficile il formarsi di una corretta opinione da parte della cittadinanza, specialmente i tanti "non addetti ai lavori".

In realtà i social network possono anche essere una preziosa fonte d'informazioni per raccolta dati, come ha dimostrato un gruppo di ricercatori che ha screenato il sito DXY.cn, un social network nato nel 2000 utilizzato da professionisti del settore sanitario in Cina.

La piattaforma sta fornendo una copertura in tempo reale dell'epidemia da SARS-COV-2. Attraverso i dati resi pubblici sul social, i ricercatori hanno sviluppato uno studio su oltre 500 pazienti, dimostrando come l'analisi di dati "crowdsouced" possa aiutare a ricostruire la progressione di un'epidemia in un contesto emergenziale.

Riferimento bibliografico

Sun K M et al. Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population level observational study. Lancet Digital Health 2020.

Segue articolo full text originale

Articles

Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population-level observational study

Kaiyuan Sun, Jenny Chen, Cécile Viboud

Summary

Background As the outbreak of coronavirus disease 2019 (COVID-19) progresses, epidemiological data are needed to guide situational awareness and intervention strategies. Here we describe efforts to compile and disseminate epidemiological information on COVID-19 from news media and social networks.

Methods In this population-level observational study, we searched DXY.cn, a health-care-oriented social network that is currently streaming news reports on COVID-19 from local and national Chinese health agencies. We compiled a list of individual patients with COVID-19 and daily province-level case counts between Jan 13 and Jan 31, 2020, in China. We also compiled a list of internationally exported cases of COVID-19 from global news media sources (Kyodo News, The Straits Times, and CNN), national governments, and health authorities. We assessed trends in the epidemiology of COVID-19 and studied the outbreak progression across China, assessing delays between symptom onset, seeking care at a hospital or clinic, and reporting, before and after Jan 18, 2020, as awareness of the outbreak increased. All data were made publicly available in real time.

Findings We collected data for 507 patients with COVID-19 reported between Jan 13 and Jan 31, 2020, including 364 from mainland China and 143 from outside of China. 281 (55%) patients were male and the median age was 46 years (IQR 35–60). Few patients (13 [3%]) were younger than 15 years and the age profile of Chinese patients adjusted for baseline demographics confirmed a deficit of infections among children. Across the analysed period, delays between symptom onset and seeking care at a hospital or clinic were longer in Hubei province than in other provinces in mainland China and internationally. In mainland China, these delays decreased from 5 days before Jan 18, 2020, to 2 days thereafter until Jan 31, 2020 (p=0·0009). Although our sample captures only 507 (5·2%) of 9826 patients with COVID-19 reported by official sources during the analysed period, our data align with an official report published by Chinese authorities on Jan 28, 2020.

Interpretation News reports and social media can help reconstruct the progression of an outbreak and provide detailed patient-level data in the context of a health emergency. The availability of a central physician-oriented social network facilitated the compilation of publicly available COVID-19 data in China. As the outbreak progresses, social media and news reports will probably capture a diminishing fraction of COVID-19 cases globally due to reporting fatigue and overwhelmed health-care systems. In the early stages of an outbreak, availability of public datasets is important to encourage analytical efforts by independent teams and provide robust evidence to guide interventions.

Funding Fogarty International Center, US National Institutes of Health.

Lancet Digital Health 2020

Published Online
February 20, 2020
https://doi.org/10.1016/S2589-7500(20)30026-1

See Online/Comment
https://doi.org/10.1016/S2589-7500(20)30055-8

Division of International Epidemiology and Population Studies, Fogarty International Center, US National Institutes of Health, Bethesda MD, USA (K Sun PhD, J Chen BSc, C Viboud PhD)

Correspondence to:
Dr Cécile Viboud, Division of International Epidemiology and Population Studies, Fogarty International Center, US National Institutes of Health, Bethesda, MD 20892, USA
viboudc@mail.nih.gov

Introduction

As the outbreak of coronavirus disease 2019 (COVID-19) is rapidly expanding in China and beyond, with the potential to become a worldwide pandemic,[1] real-time analyses of epidemiological data are needed to increase situational awareness and inform interventions.[2] Previously, real-time analyses have shed light on the transmissibility, severity, and natural history of an emerging pathogen in the first few weeks of an outbreak, such as with severe acute respiratory syndrome (SARS), the 2009 influenza pandemic, and Ebola.[3–6] Analyses of detailed line lists of patients are particularly useful to infer key epidemiological parameters, such as the incubation and infectious periods, and delays between infection and detection, isolation, and reporting of cases.[3,4] However, official individual patient data rarely become publicly available early on in an outbreak, when the information is most needed.

Building on our previous experience collating news reports to monitor transmission of Ebola virus,[7] here we present an effort to compile individual patient information and subnational epidemic curves on COVID-19 from a variety of online resources. Data were made publicly available in real time and were used by the infectious disease modelling community to generate and compare epidemiological estimates relevant to interventions. We describe the data generation process and provide an early analysis of age patterns of COVID-19,

Research in context

Evidence before this study

An outbreak of coronavirus disease 2019 (COVID-19) was recognised in early January, 2020, in Wuhan City, Hubei province, China. The new virus is thought to have originated from an animal-to-human spillover event linked to seafood and live-animal markets. The infection has spread locally in Wuhan and elsewhere in China, despite strict intervention measures implemented in the region where the infection originated on Jan 23, 2020. More than 500 patients infected with COVID-19 outside of mainland China have been reported between Jan 1 and Feb 14, 2020. Although laboratory testing for COVID-19 quickly ramped up in China and elsewhere, information on individual patients remains scarce and official datasets have not been made publicly available. Patient-level information is important to estimate key time-to-delay events (such as the incubation period and interval between symptom onset and visit to a hospital), analyse the age profile of infected patients, reconstruct epidemic curves by onset dates, and infer transmission parameters. We searched PubMed for publications between Jan 1, 1990, and Feb 6, 2020, using combinations of the following terms: ("coronavirus" OR "2019-nCoV") AND ("line list" OR "case description" OR "patient data") AND ("digital surveillance" OR "social media" OR "crowd-sourced data"). The search retrieved one relevant study on Middle East respiratory syndrome coronavirus that mentioned FluTrackers in their discussion, a website that aggregates epidemiological information on emerging pathogens. However, FluTrackers does not report individual-level data on COVID-19.

Added value of this study

To our knowledge, this is the first study that uses crowdsourced data from social media sources to monitor the COVID-19 outbreak. We searched DXY.cn, a Chinese health-care-oriented social network that broadcasts information from local and national health authorities, to reconstruct patient-level information on COVID-19 in China. We also queried international media sources and national health agency websites to collate data on international exportations of COVID-19. We describe the demographic characteristics, delays between symptom onset, seeking care at a hospital or clinic, and reporting for 507 patients infected with COVID-19 reported until Jan 31, 2020. The overall cumulative progression of the outbreak is consistent between our line list and an official report published by the Chinese national health authorities on Jan 28, 2020. The estimated incubation period in our data aligns with that of previous work. Our dataset was made available in the public domain on Jan 21, 2020.

Implications of all the available evidence

Crowdsourced line-list data can be reconstructed from social media data, especially when a central resource is available to curate relevant information. Public access to line lists is important so that several teams with different expertise can provide their own insights and interpretations of the data, especially in the early phase of an outbreak when little information is available. Publicly available line lists can also increase transparency. The main issue with the quality of patient-level data obtained during health emergencies is the potential lack of information from locations overwhelmed by the outbreak (in this case, Hubei province and other provinces with weaker health infrastructures). Future studies based on larger samples of patients with COVID-19 could explore in more detail the transmission dynamics of the outbreak in different locations, the effectiveness of interventions, and the demographic factors driving transmission.

case counts across China and internationally, and delays between symptom onset, admissions to hospital, and reporting, for cases reported until Jan 31, 2020.

Methods

Study design and Chinese data sources

For **DXY website** see DXY.cn

In this population-level observational study, we used crowdsourced reports from DXY.cn, a social network for Chinese physicians, health-care professionals, pharmacies, and health-care facilities established in 2000. This online platform is providing real-time coverage of the COVID-19 outbreak in China, obtained by collating and curating reports from news media, government television, and national and provincial health agencies. The information reported includes time-stamped cumulative counts of COVID-19 infections, outbreak maps, and real-time streaming of health authority announcements in Chinese (directly or through state media).[8] Every report is linked to an online source, which can be accessed for more detailed information on individual cases.

For **an example of an online source** see https://ncov.dxy.cn/ncovh5/view/pneumonia

These are publicly available, de-identified patient data reported directly by public health authorities or by state media. No patient consent was needed and no ethics approval was required.

Data compilation

We closely monitored updates on DXY.cn between Jan 20, 2020, and Jan 31, 2020, to extract key information on individual patients in near real-time, and reports of daily case counts. For individual-level patient data, we used descriptions from the original source in Chinese to retrieve age, sex, province of identification, travel history, reporting date, dates of symptom onset and seeking care at a hospital or clinic, and discharge status, when available. Individual-level patient data were formatted into a line-list database for further quantitative analysis. Individual-level patient data were entered from DXY.cn by a native Chinese speaker (KS), who also generated an English summary for each patient. Entries were checked by a second person (JC). Since DXY.cn primarily provides

information on patients reported in China, we also compiled additional information on internationally exported cases of COVID-19. We obtained data for 21 countries outside of mainland China (Australia, Cambodia, Canada, France, Germany, Hong Kong, India, Italy, Japan, Malaysia, Nepal, Russia, Singapore, South Korea, Sri Lanka, Taiwan, Thailand, United Arab Emirates, the UK, the USA, and Vietnam). We gathered and cross-checked data for infected patients outside of China using several sources, including global news media (Kyodo News, Straits Times, and CNN), official press releases from each country's Ministry of Health, and disease control agencies.

In addition to detailed information on individual patients, we reconstructed the daily progression of reported patients in each province of China from Jan 13, until Jan 31, 2020. We used the daily outbreak situation reports communicated by provincial health authorities, covered by state television and media, and posted on DXY.cn. All patients in our databases had a laboratory confirmed SARS coronavirus 2 (SARS-CoV-2) infection.

Our COVID-19 database was made publicly available as a Google Sheet, disseminated via Twitter on Jan 21, 2020, and posted on the website of Northeastern University, (Boston, MA, USA) on Jan 24, 2020, where it is updated in real time. Data used in this analysis, frozen at Jan 31, 2020, are available online as a spreadsheet.

Statistical analysis

We assessed the age distribution of all patients with COVID-19 by discharge status. We adjusted the age profile of Chinese patients by the population of China. We used 2016 population estimates from the Institute for Health Metrics and Evaluation[9] to calculate the relative risk (RR) of infection with COVID-19 by age group. To calculate the RR, we followed the method used by Lemaitre and colleagues[10] to explore the age profile of influenza, where RR for age group i is defined as

$$RR_i = \frac{\left(\frac{C_i}{\sum_i C_i}\right)}{\left(\frac{N_i}{\sum_i N_i}\right)}$$

where C_i is the number of cases in age group i and N_i is the population size of age group i.

To estimate trends in the strength of case detection and interventions, we analysed delays between symptom onset and visit to a health-care provider, at a hospital or clinic, and from seeking care at a hospital or clinic to reporting, by time period and location. We considered the period before and after Jan 18, 2020, when media attention and awareness of the outbreak became more pronounced.[11] We used non-parametric tests to assess differences in delays between seeking care at a hospital or clinic and reporting between locations (Wilcoxon test to compare two locations and Kruskall–Wallis test to compare three or more locations).

We estimated the duration of the incubation period on the basis of our line list data. We analysed a subset of patients returning from Wuhan who had spent less than a week in Wuhan, to ensure a narrowly defined exposure window. The incubation period was estimated as the midpoint between the time spent in Wuhan and the date of symptom onset.

We did all analyses in R (version 3.5.3). We considered p values of less than 0·05 to be significant.

Role of the funding source

The funder had no role in study design, data compilation, data analysis, data interpretation, or writing of the report. All authors had access to the data, and had final responsibility for the decision to submit for publication.

Results

Our line list comprised 507 patients reported from Jan 13, to Jan 31, 2020, including 364 (72%) from mainland China and 143 (28%) from outside of China (table). Our sample captured 5·2% of 9826 COVID-19 cases reported by WHO on Jan 31, 2020. The sex ratio was skewed towards males. In mainland China, five of 30 provinces were represented, with 133 (26%) patients reported by

	Patients (n=507)
Age, years	46 (35–60)
Sex	
Male	281 (55%)
Female	201 (40%)
Data missing	25 (5%)
Location	
Mainland China	364 (72%)
Beijing	133 (26%)
Shaanxi	87 (17%)
Hubei*	41 (8%)
Tianjin	22 (4%)
Yunnan	19 (4%)
International cases, reported outside of mainland China	143 (28%)
Relation to Wuhan	
Visited Wuhan	153 (30%)
Resident of Wuhan	152 (30%)
None	80 (16%)
Unknown†	122 (24%)
Disease outcome: death at time of reporting	40 (8%)

Data are median (IQR) or n (%). Data are publicly available on the Laboratory for the Modeling of Biological + Socio-technical systems website and on our frozen spreadsheet. COVID-19=coronavirus disease 2019. *Including 32 from Wuhan. †All patients with unknown relation to Wuhan were reported by Beijing Municipal Health Commission, Beijing, China.

Table: **Characteristics of patients with COVID-19 included in the crowdsourced line list**

For the **WHO situation report as of Jan 31, 2020,** see https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200131-sitrep-11-ncov.pdf?sfvrsn=de7c0f7_4

For the **Laboratory for the Modeling of Biological + Socio-technical systems website at Northeastern University** see https://www.mobs-lab.org/2019ncov.html

For the **spreadsheet of patient-level data until Jan 31, 2020,** see https://docs.google.com/spreadsheets/d/1Gb5cyg0fjUtsqh3hl_L-C5A23zIOXmWH5veBklfSHzg/edit?usp=sharing

Beijing, 87 (17%) by Shaanxi, 41 (8%) by Hubei (capital city is Wuhan), 19 (4%) by Tianjin, and 22 (4%) by Yunnan. Of 435 patients with known relation to Wuhan city, most reported a travel history to the city (135 [30%]) or were residents of the city (152 [30%]), while 80 (16%) had no direct relation to the city. 122 (24%) patients, all reported in Beijing, had no information about their recent history with Wuhan.

The age distribution of COVID-19 cases was skewed towards older age groups with a median age of 45 years (IQR 33–56) for patients who were alive or who had an unknown outcome at the time of reporting (figure 1). The median age of patients who had died at the time of reporting was 70 years (IQR 65–81). Few patients (13 [3%]) were younger than 15 years. Adjustment for the age demographics of China confirmed a deficit of infections among children, with a RR below 0·5 in patients younger than 15 years (figure 1). The RR measure indicated a sharp increase in the likelihood of reported COVID-19 among people aged 30 years and older.

See Online for appendix

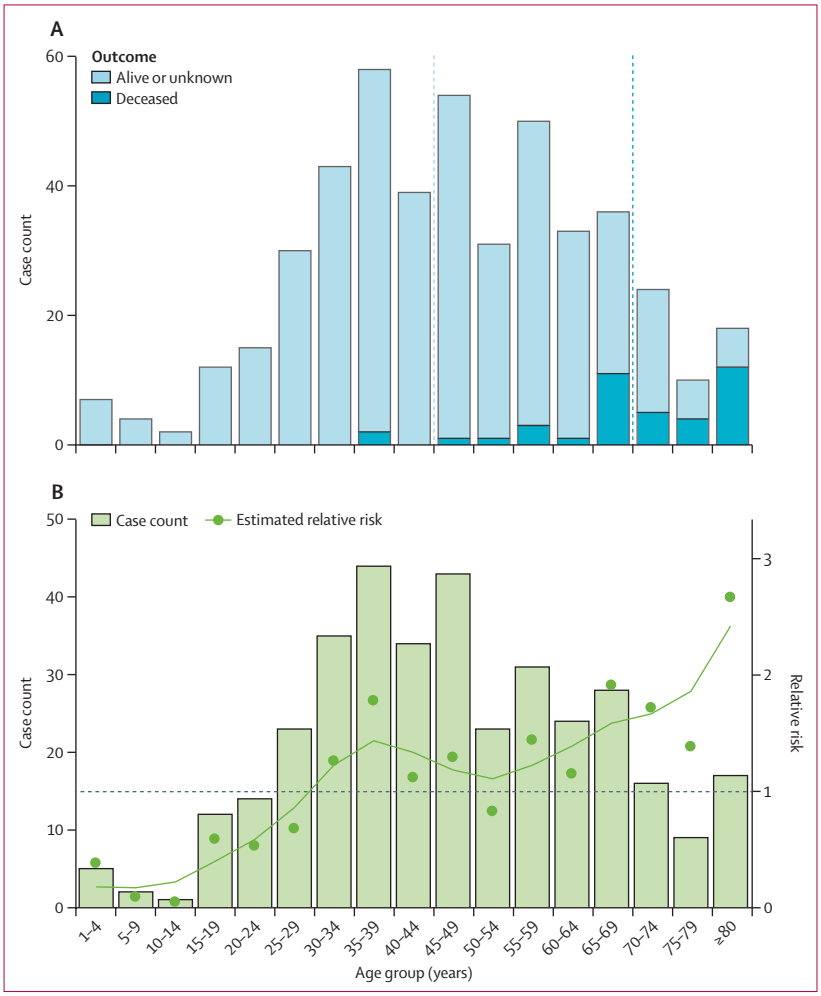


Figure 1: **Age distribution of patients with COVID-19 from crowdsourced data**
(A) All 507 cases by disease outcome (alive or unknown or deceased at time of reporting); vertical bars are case counts in each age group and the dotted lines show the median age for patients who were alive or with unknown outcomes at the time of reporting and those who had died at the time of reporting. (B) Relative risk by 5-year age band for 364 cases reported in China. The observed data are shown by bars and the estimated relative risk is shown by datapoints and a spline-smoothed curve. COVID-19=coronavirus disease 2019.

A timeline of cases in our crowdsourced patient line list is shown by date of onset in figure 2, indicating an acceleration of reported cases by Jan 13, 2020. The outbreak progression based on the crowdsourced patient line list was consistent with the timeline published by China Center for Disease Control and Prevention (CDC) on Jan 28, 2020,[12] which is based on a more comprehensive database of more than 6000 patients with COVID-19. Since Jan 23, 2020, the cumulative number of cases has slowed down in the crowdsourced and China CDC curves (figure 2), which probably reflects the delay between disease onset and reporting. The median reporting delay was 5 days (IQR 3–8) in our data.

Province-level epidemic curves are shown by reporting date in figure 3. As of Jan 31, 2020, 16 (52%) of 30 provinces in mainland China had reported more than 100 confirmed cases. The apparent rapid growth of newly reported cases between Jan 18, and Jan 31, 2020, in several provinces outside of Hubei province is consistent with sustained local transmission.

Across the study period, the median delay between symptom onset and seeking care at a hospital or clinic was 2 days (IQR 0–5 days) in mainland China (figure 4). This delay decreased from 5 days before Jan 18, 2020, to 2 days thereafter (Wilcoxon test p=0·0009). Some provinces, such as Tianjin and Yunnan had shorter delays (data by province not shown), while the early cases from Hubei province were characterised by longer delays in seeking care (median 0 days [IQR 0–1]).

The median delay between seeking care at a hospital or clinic and reporting was 2 days (IQR 2–5 days) in mainland China and decreased from 9 days before Jan 18, 2020, to 2 days thereafter (Wilcoxon test p<0·0001; figure 4). Similarly to delays in seeking care at a hospital or clinic, reporting was quickest in Tianjin and Yunnan (median 1 day [IQR 0–1]) and slowest in Hubei province (median 12 days [IQR 7–16]).

The median delay between symptom onset and seeking care at a hospital or clinic was 1 day (IQR 0–3) for international travellers, and shorter than for patients in Hubei province or the rest of mainland China (Kruskal–Wallis test p<0·0001; figure 4). Even in the period after Jan 18, 2020, when awareness of the outbreak increased, a shorter delay between symptom onset and seeking care at a hospital or clinic was seen for international patients than for those in mainland China (Wilcoxon test p<0·0001). For international cases, the delay between seeking care at a hospital or clinic and reporting was 2 days (IQR 1–4), also shorter than for mainland China (Wilcoxon test p<0·0001; figure 4).

On the basis of 33 patients with a travel history to Wuhan, we estimated the median incubation period for COVID-19 to be 4·5 days (IQR 3·0–5·5; appendix p 2).

Discussion

Information from patient line lists is crucial but difficult to obtain at the beginning of an outbreak. Here we have shown that careful compilation of crowdsourced reports curated by a long-standing Chinese medical social network provides a valuable picture of the outbreak of COVID-19 in real time. The outbreak timeline is consistent with aggregated case counts provided by health authorities. For comparison, China CDC published the first epidemic curve by symptom onset on Jan 28, 2020.[12] Line lists provide unique information on the delays between symptom onset and detection by the health-care system, reporting delays, and travel histories. This information cannot be extracted from aggregated case counts published by official sources. Line list data can help assess the effectiveness of interventions and the potential for widespread transmission beyond the initial foci of infection. In particular, shorter delays between symptom onset and admission to hospital or seeking care in a hospital or clinic accelerate detection and isolation of cases, effectively shortening the infectious period.

A useful feature of our crowdsourced database was the availability of travel histories for patients returning from Wuhan, which, along with dates of symptom onset, allowed for estimation of the incubation period here and in related work.[13,14] A narrow window of exposure could be defined for a subset of patients who had a short stay in Wuhan, at a time when the epidemic was still localised to Wuhan. Several teams have used our dataset and datasets from others to estimate a mean incubation period for COVID-19 to be 5–6 days (95% CI 2–11).[13–16] Our own estimate (median 4·5 days [IQR 3·0–5·5]) is consistent with previous work that used other modelling approaches.[13–16] The incubation period is a useful parameter to guide isolation and contact tracing; based on existing data, the disease status of a contact should be known with near certainty after a period of observation of 14 days.[13] Availability of a public dataset enables independent estimation of important epidemiological parameters by several teams, allowing for confirmation and cross-checking at a time when information can be conflicting and noisy.

An interesting finding in our data relates to the age distribution of patients. We found a heavy skew of infection towards older age groups, with substantially fewer children infected. This pattern could indicate age-related differences in susceptibility to infection, severe outcomes, or behaviour. However, a substantial portion of the patients in our database are travellers, a population that is usually predominantly adults (although does not exclude children). Furthermore, because patient data in our dataset were captured by the health system, they are biased towards the more severe spectrum of the disease, especially for patients from mainland China. Clinical reports have shown that severity of COVID-19 is associated with the presence of chronic conditions,[16,17] which are more frequent in older age groups. Nevertheless, we would also expect children younger than 5 years to be at risk of severe outcomes and to be reported to the health-care system, as is seen for other respiratory infections.[18]

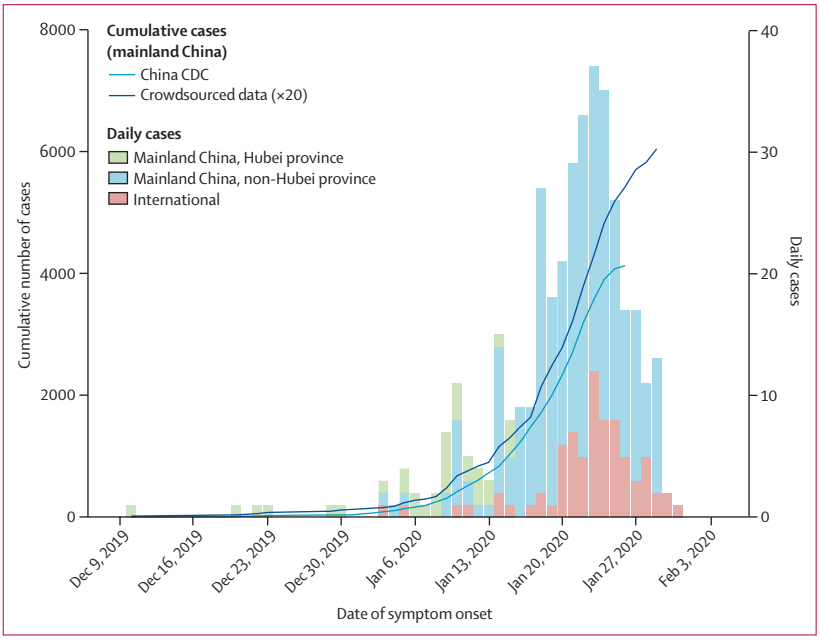


Figure 2: **Daily timeline of the COVID-19 epidemic based on crowdsourced data and official sources, by location**
All data are by date of symptom onset. Cumulative curves are shown for the official China CDC data (published on Jan 28, 2020), and for the crowdsourced data. Crowdsourced data have been rescaled and multiplied by 20 to enable clear comparison with the official China CDC data. Histograms are daily case count, based on crowdsourced data for Hubei province, mainland China non-Hubei province, and cases outside of mainland China. CDC=Centers for Disease Control. COVID-19=coronavirus disease 2019.

Biological differences could have a role in shaping these age profiles. A detailed analysis of one of the early COVID-19 clusters by Chan and colleagues[19] revealed symptomatic infections in five adult members of the same household, while a child in the same household aged 10 years was infected but remained asymptomatic, potentially indicating biological differences in the risk of clinical disease driven by age. Previous immunity from infection with a related coronavirus has been speculated to potentially protect children from SARS,[20,21] and so might also have a role in COVID-19. In any case, if the age distribution of cases reported here was to be confirmed and the epidemic were to progress globally, we would expect an increase in respiratory mortality concentrated among people aged 30 years and older. This mortality pattern would be substantially different from the profile of the 2009 influenza pandemic, for which excess mortality was concentrated in those younger than 65 years.[21]

In our dataset, we saw a rapid increase in the number of people infected with COVID-19 in several provinces of China, consistent with local transmission outside of Hubei province. As of Jan 31, 2020, province-level epidemic curves are only available by date of reporting, rather than date of symptom onset, which usually inflates recent case counts if detection has increased.

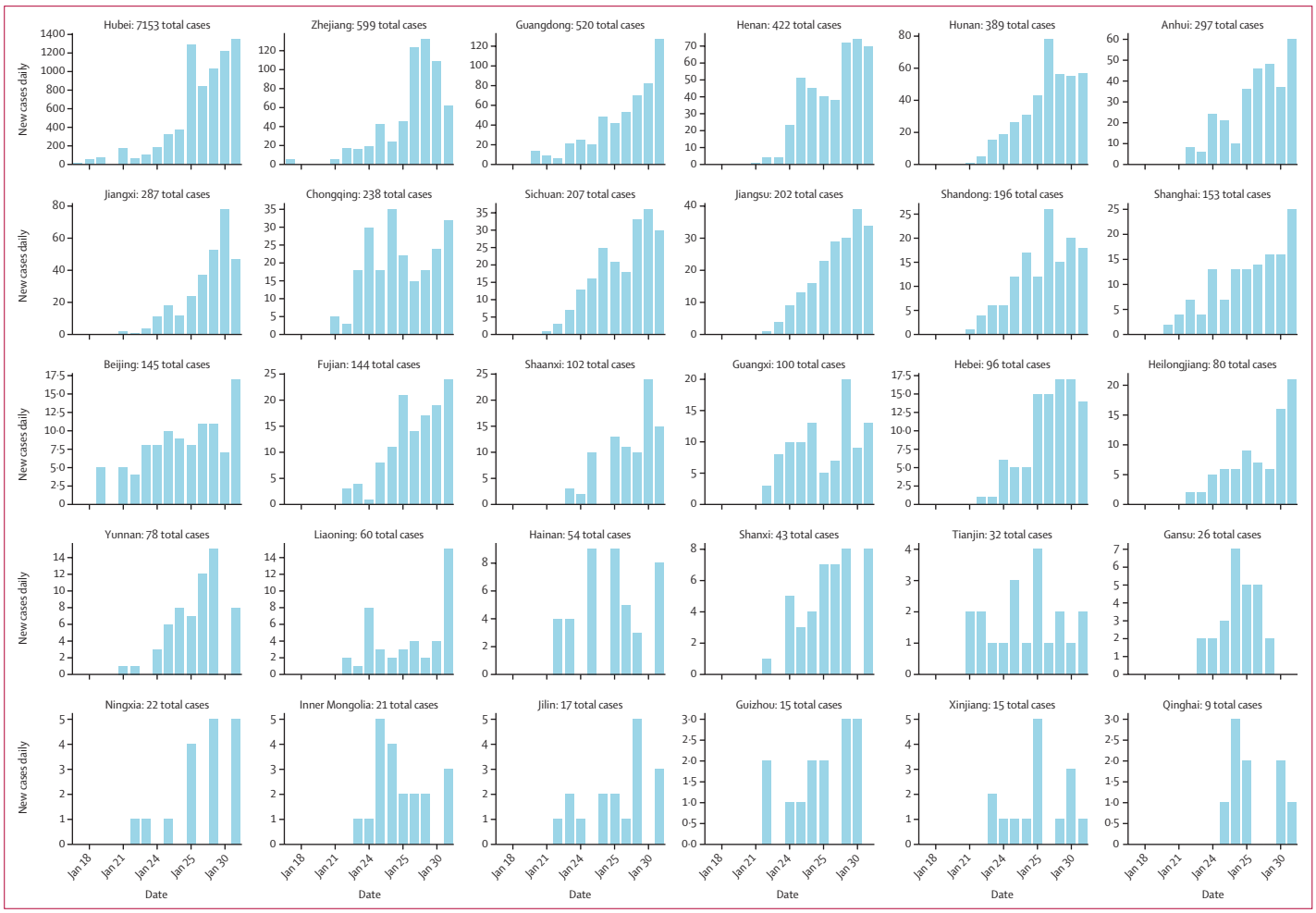


Figure 3: **Daily timeline of the COVID-19 epidemic at the provincial level in China, during January, 2020**
Vertical bars show the daily counts of new reported cases, with provinces sorted by total number of reported cases. The timeline for each province is reconstructed on the basis of daily outbreak situation reports provided by provincial health authorities and posted on DXY.cn and are true as of Jan 31, 2020. COVID-19=coronavirus disease 2019.

Furthermore, province-level data include both returning travellers from Hubei province (ie, importations) and locally acquired cases, which also usually inflate the apparent risk of local transmission. Notably, other lines of evidence suggest that local transmission is now well established outside of Hubei province, because travel increased just before the Chinese New Year on Jan 25, 2020, and before implementation of the travel ban in Wuhan.[22] Accordingly, our own data include evidence of transmission clusters in non-travellers, with, for instance, a second-generation transmission event reported in Shaanxi on Jan 21, 2020.

Our study had several limitations, one of which was the data we used. Although all provinces in mainland China provide aggregated information on infections and deaths, individual-level patient descriptions are only available for a subset of provinces. Geographical coverage is heterogeneous in our line list, and we have a notable deficit of cases from Hubei province, the foci of the COVID-19 outbreak. We expect that little patient-level information is shared on social media by province-level and city-level health authorities in Wuhan and Hubei province because health systems are overwhelmed. For similar reasons, provinces with a large total case count at the end of January, 2020, or with a weaker health infrastructure, were under-represented in our line list, with the exception of Beijing. Other limitations in our data include severity (only patients who had severe enough symptoms to seek care were captured) and changes in case definition. A series of epidemiological criteria were required for COVID-19 testing, including travel history to Wuhan within the past 2 weeks; residence in Wuhan within the past 2 weeks; contact with individuals from Wuhan (with fever and respiratory symptoms) within the past 2 weeks; and being part of an established disease cluster. Some of these criteria (eg, relation to Wuhan) were relaxed over time (appendix). As a result, we have an over-representation of travel-related cases in our database.

The reproduction number is an important quantity for outbreak control. We refrained from estimating this

parameter because reporting changes could bias estimates relying on epidemic growth rates. Furthermore, our dataset captured cases all over China and does not reflect transmission patterns in any particular location. A mean reproduction number of 2·5–2·7 has previously been estimated on the basis of the volume of importations of international cases in the pre-intervention period in Wuhan.[11]

We recognise that, although our data source is useful and timely, it should not replace official statistics. Manual compilation of detailed line lists from media sources is highly time consuming and is not sustainable when case counts reach several thousands. Here we provide detailed data on 507 patients when the official case count was over 9000 by Jan 31, 2020, representing a sample of approximately 5% of reported cases and a much smaller proportion of the full spectrum of COVID-19 cases, which include mild infections. A crowdsourced system would not be expected to catch all cases, especially if many cases are too mild to be captured by the health-care system, digital surveillance, or social media. Notably, DXY.cn does not generate data outside of traditional surveillance systems but rather provides a channel of rapid communication between the public and health authorities. In turn, our approach has helped extract and repackage information from health authorities into an analytical format, which was not available elsewhere.

At the time of writing, efforts are underway to coordinate compilation of COVID-19 data from online sources across several academic teams. Ultimately, we expect that a line list of patients will be shared by government sources with the global community; however, data cleaning and access issues might take a prohibitively long time to resolve. For the west African Ebola outbreak, a similarly coordinated effort to publish a line list took 2 years.[23] Given the progression of the COVID-19 outbreak, such a long delay would be counterproductive.

Overall, the novelty of our approach was to rely on a unique source for social media and news reports in China, which aggregated and curated relevant information. This approach facilitated entry of robust and standard data on clinical and demographic information. Reassuringly, DXY.cn maintains a special section dedicated to debunking fake news, myths, and rumours about the COVID-19 outbreak. Looking to the future, collection of patient data in the context of emergencies could include information on whether patients are identified through contact tracing or because they seek care on their own. Furthermore, data interpretability could be improved by gathering more quantitative information on how case definitions are used in practice.

In conclusion, crowdsourced epidemiological data can be useful to monitor emerging outbreaks, such as COVID-19 and, as previously, Ebola virus.[7] These efforts can help generate and disseminate detailed information in the early stages of an outbreak when little other data are available, enabling independent estimation of key parameters that affect interventions. Based on our small sample of patients with COVID-19, we note an intriguing age distribution, reminiscent of that of SARS, which warrants further epidemiological and serological studies. We also report early signs that the response is strengthening in China on the basis of a decrease in case detection time, and rapid management of travel-related infections that are identified internationally. This is an early report of a rapidly evolving situation and the parameters discussed here could change quickly. In the coming weeks, we will continue to monitor the epidemiology of this outbreak using data from news reports and official sources.

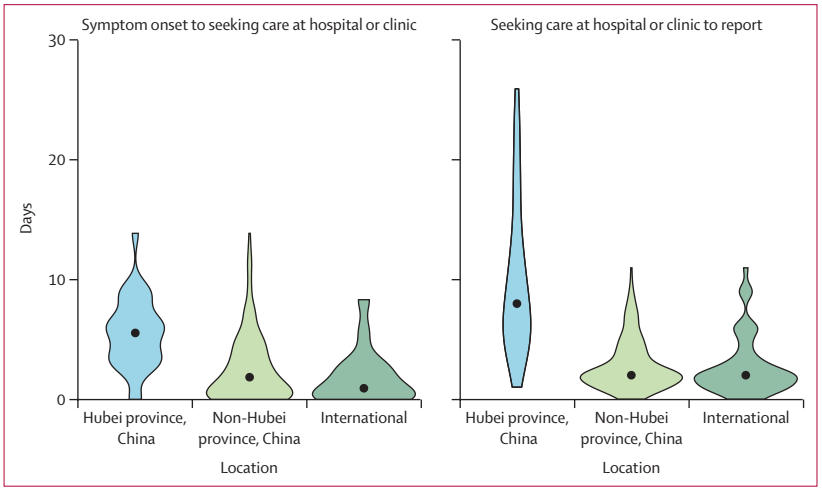


Figure 4: **Delay between symptom onset and seeking care at a hospital or clinic (A) and between seeking care at a hospital or clinic and reporting (B) of COVID-19 cases, by location**
Data are for the entire study period and include all cases reported between Jan 13 and Jan 31, 2020. Datapoints are medians, with the spread of data indicated by the filled shapes. All time intervals significantly differ between locations (Kruskall Wallis test, p<0·0001). COVID-19=coronavirus disease 2019.

Contributors
KS and CV contributed to the study design. KS and JC contributed to the data compilation. KS, JC, and CV contributed to data analysis. KS and JC contributed to the design and drawing of figures. KS, JC, and CV contributed to the writing of the manuscript.

Declaration of interests
We declare no competing interests.

Data sharing
All data used in this report have been made publicly available on the Laboratory for the Modeling of Biological + Socio-technical systems website of Northeastern University. The available data include daily case counts of COVID-19 by reporting date and Chinese province, and a de-identified line list of patients with COVID-19. The line list includes geographical location (country and province), reporting date, dates of symptom onset and seeking care at a hospital or clinic, relation to Wuhan, discharge status when known, an English summary of the case description from media sources, and a link to the original source of data.

Acknowledgments
The study was funded by the in-house research division of the Fogarty International Center. CV and KS acknowledge support from the Bill & Melinda Gates Foundation. The findings and conclusions in this study are those of the authors and do not necessarily represent the official position of the US National Institutes of Health or US Department of Health and Human Services.

References

1 WHO. Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). Geneva: World Health Organization, 2020. https://www.who.int/news-room/detail/30–01–2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov) (accessed Feb 10, 2020).

2 Rivers C, Chretien JP, Riley S, et al. Using "outbreak science" to strengthen the use of models during epidemics. *Nat Commun* 2019; **10:** 3102.

3 Chowell G, Bertozzi SM, Colchero MA, et al. Severe respiratory disease concurrent with the circulation of H1N1 influenza. *N Engl J Med* 2009; **361:** 674–79.

4 Chowell G, Echevarría-Zuno S, Viboud C, et al. Characterizing the epidemiology of the 2009 influenza A/H1N1 pandemic in Mexico. *PLoS Med* 2011; **8:** e1000436.

5 Fraser C, Donnelly CA, Cauchemez S, et al. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science* 2009; **324:** 1557–61.

6 Lipsitch M, Cohen T, Cooper B, et al. Transmission dynamics and control of severe acute respiratory syndrome. *Science* 2003; **300:** 1966–70.

7 Cleaton JM, Viboud C, Simonsen L, Hurtado AM, Chowell G. Characterizing Ebola transmission patterns based on internet news reports. *Clin Infect Dis* 2016; **62:** 24–31.

8 DXY.cn. Pneumonia. 2020. http://3g.dxy.cn/newh5/view/pneumonia (accessed Feb 10, 2020; in Chinese).

9 Institute for Health Metrics and Evaluation. Global health data exchange. Seattle, WA: Institute for Health Metrics and Evaluation, 2020. ghdx.healthdata.org (accessed Feb 14, 2020).

10 Lemaitre M, Carrat F. Comparative age distribution of influenza morbidity and mortality during seasonal influenza epidemics and the 2009 H1N1 pandemic. *BMC Infect Dis* 2010; **10:** 162.

11 MRC Centre for Global Infectious Disease Analysis. News/2019-nCoV. London: Imperial College London, 2020. https://www.imperial.ac.uk/mrc-global-infectious-disease-analysis/news--wuhan-coronavirus/ (accessed Feb 10, 2020).

12 China Center for Disease Control and Prevention. 2019 epidemic update and risk assessment of 2019 novel coronavirus. Beijing: China Center for Disease Control and Prevention, 2020. http://www.chinacdc.cn/yyrdgz/202001/P020200128523354919292.pdf (accessed Feb 10, 2020).

13 Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. *Euro Surveill* 2020; **25:** 2000062.

14 Lauer SA, Grantz KH, Bi Q, et al. The incubation period of 2019-nCoV from publicly reported confirmed cases: estimation and application. *medRxiv* 2020; published online Feb 4. DOI:10.1101/2020.02.02.20020016 (preprint).

15 Zhao A, Ran J, Musa SS, et al. Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: a data-driven analysis in the early phase of the outbreak. *bioRxiv* 2020; published online Jan 24. DOI:10.1101/2020.01.23.916395v1 (preprint)

16 Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* 2020; published online Jan 29. DOI:10.1056/NEJMoa2001316.

17 Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; published online Jan 24. https://doi.org/10.1016/S0140-6736(20)30183-5.

18 Greenbaum AH, Chen J, Reed C, et al. Hospitalizations for severe lower respiratory tract infections. *Pediatrics* 2014; **134:** 546–54.

19 Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020; published online Jan 24. https://doi.org/10.1016/S0140-6736(20)30154-9.

20 Leung GM, Hedley AJ, Ho LM, et al. The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: an analysis of all 1755 patients. *Ann Intern Med* 2004; **141:** 662–73.

21 Simonsen L, Spreeuwenberg P, Lustig R, et al. Global mortality estimates for the 2009 Influenza Pandemic from the GLaMOR project: a modeling study. *PLoS Med* 2013; **10:** e1001558.

22 Du W, Wang L, Cauchemez S, et al. Risk of 2019 novel coronavirus importations throughout China prior to the Wuhan quarantine *medRxiv* 2020; published online Feb 4. DOI:10.1101/2020.01.28.20019299 (preprint).

23 Agua-Agum J, Ariyarajah A, Aylward B, et al. Exposure patterns driving ebola transmission in west Africa: A retrospective observational study. *PLoS Med* 2016; **13:** e1002170.

Cod. IT/SOL-PNH/17/0045

Appendice

Springer Healthcare Education

ebookecm.it

Altri materiali in italiano di libera fruizione

Infografiche ISS

Glossario ISS sulle parole chiave dell'epidemia arricchito dei termini "mortalità" e "letalità"

Schede di autovalutazione SIMIT (Società Italiana Malattie Infettive)

Scheda di valutazione Medico di medicina generale FIMMG

Misure organizzative volte al contenimento e gestione dell'emergenza epidemiologica derivante da COVID-19 – Regione Campania

COVID-2019. Nuove indicazioni e chiarimenti – Circolare Ministero Salute 22/02/19

Aggiornamento emotrasfusioni nuovo coronavirus

Documento relativo ai criteri per sottoporre soggetti clinicamente asintomatici alla ricerca d'infezione da SARS-CoV-2 attraverso tampone rino-faringeo e test diagnostico (A cura del Gruppo di lavoro permanente costituito nell'ambito del Consiglio Superiore di Sanità)

Documento relativo alla definizione di "Paziente guarito da Covid-19" e di "Paziente che ha eliminato il virus SARS-CoV-2" (Ministero Salute)

Definizione di caso di COVID-19 per la segnalazione (Ministero Salute)

Rapporto tecnico ECDC (Traduzione SITI) Personal protective equipment (PPE) needs in healthcare settings for the care

Rapporto tecnico ECDC (Traduzione SITI) Infection prevention and control for the care of patients with 2019-nCoV in healthcare settings

Traduzione ad opera di Faster dell'articolo pubblicato su Journal of the American College of Radiology: A Coronavirus Outbreak: What The Department of Radiology Should Know