Letters to the Editor

Emergence of a novel coronavirus causing respiratory illness from Wuhan, China

Dear Editor,

In previous reports, workers have characterized the presentation of Middle East Respiratory Syndrome (MERS)[1] and Severe Acute Respiratory Syndrome (SARS)[2] to aid clinical teams in the recognition, diagnosis and management of these cases. Now with the emergence of a novel coronavirus (CoV) from Wuhan, China (tentatively named as 2019-nCoV by The World Health Organization – WHO),[3,4] similar clinical, diagnostic and management guidance are required.

Available information at the time of writing indicates that the virus has now spread beyond China and infection has been confirmed in individuals without direct contact with the index Wuhan wet market (Huanan South China Seafood Market), where the sale of game meat from live animals was also available. This suggests that human-to-human transmission is not only possible but very likely. A full genome phylogenetic analysis of this 2019-nCoV indicates that it is closely related to bat SARS-like CoV (Fig. 1), compatible with a zoonotic origin for this virus, similar to SARS-CoV and MERS-CoV.[5]

Two cases have now been confirmed to date in Thailand. The first was a 61-year old Chinese woman travelling with 5 family members in a 16-member tour group. She developed symptoms of fever, chills, headache and sore throat on 5 January 2020 and flew from Wuhan directly to Thailand on 8 January, where she was diagnosed and isolated. She reported regular visits to wet markets in Wuhan but not the index wet market from where most cases were reported.[6]

The second case confirmed in Thailand was that of a 74-year old Chinese woman, who was laboratory-confirmed to be infected with the 2019-nCoV on 17 January 2020. This second case was not linked epidemiologically to the first case, and she had not visited any market in Wuhan. So far both cases are recovering well in the negative pressure isolation facilities at the Bamrasnaradura Institute in Thailand, and may be discharged soon.[7,8]

In addition, one case of 2019-nCoV was confirmed in a male patient in his thirties in Japan who was staying in Wuhan during late December 2019 to early January 2020, and developed fever on 3 January. Although he had not visited any wet or live animal markets during his stay in Wuhan, he did report close contact with someone with pneumonia. On return to Japan on 6 January he visited a local clinic where he tested negative for influenza. Despite this, his symptoms of fever, cough, and sore throat continued, so he attended a local hospital on 10 January where he was admitted and found to have abnormal infiltrates on his chest X-ray. He remained febrile until 14 January and was eventually tested as positive for 2019-nCoV on 15 January.[9] He became afebrile on the same day and was discharged home where he remains stable. This was the second 2019-nCoV case to be confirmed outside of China (being identified between the two cases from Thailand).

Thus, clinically, the symptoms of 2019-nCoV infection appear very non-specific and may be very similar to influenza, including fever, cough, fatigue, sore throat, runny nose, headache and shortness of breath, with possible ground glass shadowing on the chest X-ray. Importantly, such symptoms appear to persist longer in cases of 2019-nCoV infection than in most cases of uncomplicated influenza. Similar to SARS and MERS, there is still no specific, licensed antiviral treatment for CoVs and the clinical management is mainly supportive. Infection control guidance will be likely based on existing guidance for SARS and MERS, perhaps with some additional heightened precautions due to the largely unknown nature of this new virus.

Also similar to the SARS and MERS cases, there is likely a lot of variability in the clinical presentation, including mild or asymptomatic cases that may never present to healthcare services. Larger population level seroprevalence studies to test for past infection or exposure are required to determine how many such cases may exist. Whether "super-spreaders" who are associated with multiple secondary infections, as has occurred most prominently with SARS but also MERS, will be (or indeed may have already been) a feature of the epidemiology of this virus is not yet known.

No pediatric 2019-nCoV infections have been diagnosed so far, and infections in other vulnerable patient groups, such as transplant and other immunocompromised patients, pregnant women and those with chronic diseases (diabetes, liver, kidney, heart disease, etc.) and extremes of the body-mass index (BMI), are yet to be reported. Further data are awaited on such cases.

Based on the current and limited data available and the likelihood that many milder or asymptomatic cases have not presented to healthcare services, it is too early to compare case-fatality rates with SARS or MERS. So far, there have been two deaths out of a total of 48 cases reported from Wuhan and overseas,[7] which have been in older patients with various comorbidities.

Most recently, a mathematical modelling study from Imperial College (London, UK) suggests that the number of unrecognized, undiagnosed cases could be as high as 4400–4500, though ∼1700 may be more realistic – assuming that the model assumptions are reasonably accurate.[10] This situation is evolving and more updates will be forthcoming.

SARS was the first emerging infectious disease of the 21st century and it came and went quickly despite a tremendous global impact. MERS in contrast remains largely confined to the Middle East with occasional exported cases and has smouldered since 2012. We clearly have a lot to learn about these zoonotic bat coronaviruses (see Fig. 1), but hopefully the scientific, medical and public health worlds are now much better prepared this time round to deal with this new emerging threat.
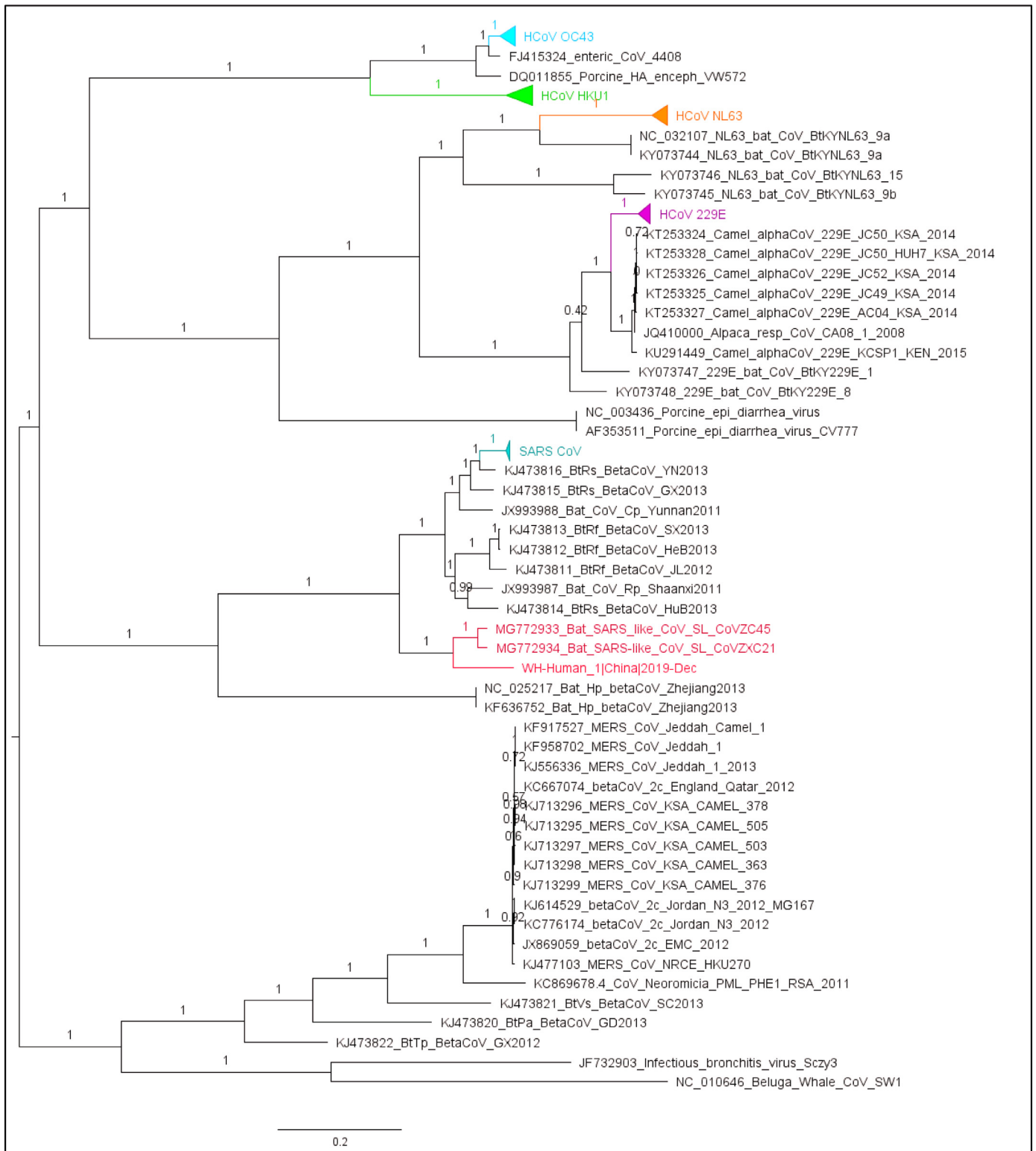
Fig. 1. A maximum likelihood tree using full genome coronavirus sequences (30–40 kbp in length) from GenBank was constructed using Fast Tree (v2.1) under a GTR model of evolution. Initial alignment was performed using the online multiple alignment program (MAFFT v.7: https://mafft.cbrc.jp/alignment/server/) with further manual editing. The final tree was displayed and annotated in FigTree v1.4.4. Large groups of similar sequences belong to HCoV OC43, HKU1, NL63, 229E ad SARS CoV have been collapsed for clarity. Figures on the branches are Shimodaira-Hasegawa-like support values, ranging from 0 to 1, with higher values indicating that the branch topologies (or 'splits') are more likely to be real. The Wuhan 2019-nCoV (GenBank Accession no. MN908947) is shown in red along with its closest relative bat SARS-like CoVs.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Genetic diversity and potential recombination between ferret coronaviruses from European and American lineages

Dear Editor,

Recently, an outbreak of unusual viral pneumonia in Wuhan city, China has sickened dozens of people. Preliminary studies indicated a novel coronavirus as the likely cause of the outbreak. Genetic recombination has been shown to contribute to the evolution of coronaviruses, including severe acute respiratory syndrome coronavirus (SARS-CoV) and the middle east respiratory syndrome coronavirus (MERS-CoV). Recent papers in this journal also described the involvement of recombination in viral evolution.[1,2] Here I use ferret coronaviruses (FRCoVs) as an example to show recombination in coronaviruses.

Coronaviruses (CoVs) are enveloped, single positive-stranded RNA viruses that can infect a wide range of host species. CoV was first reported to infect ferret and associate with epizootic catarrhal enteritis in the United States in 2006, referred to ferret enteric coronavirus (FRECV).[3] A fatal variant, ferret systemic coronavirus (FRSCV) caused feline infectious peritonitis-like disease in ferrets from Europe and the United States from 2002 to 2007.[4] To date, FRCoVs have been detected in multiple countries, including the Netherlands, the United Kingdom, Spain, the United States, Peru and Japan.[5] This study aim to assess the genetic diversity and potential role of genetic recombination in the evolutionary dynamics of FRCoVs.

Genetic analyses were conducted with five complete genomes and 160 gene sequences of FRCoVs downloaded from the NIAID Virus Pathogen Database and Analysis Resource.[6] These sequences were analysed in combination with 36 representative genomes of CoVs from other host species. Phylogenetic analysis of the complete genome confirmed the division of four genetic genera in CoVs. FRCoVs fall in alpha genera and most closely related to the CoV from mink (Fig. S1). FRCoVs from US and Japan (ferrets imported from US) were more closely related to each other than a Dutch strain. Phylogenetic tree for N gene supported two geographically dependent lineages, European and American (Fig. 1A). The European lineage comprised FRCoVs from the Netherlands and Slovenia, while the American lineage comprised FRCoVs from US and Japan. Phylogenetic tree for RdRp gene showed that the American and Japanese strains comprised the American Lineage and are distant from the European lineage represented by the Dutch strain (Fig. S2). Different grouping was observed in the phylogenetic tree for S gene. The European lineage was consistent with that observed in the N tree, however, the American lineage was further divided into two sub-groups: American-I and American-II (Fig. 1B). The American-II sub-group comprised four FRCoVs from America, but was more closely related to European lineage rather than the American-I sub-group. Differences between the topologies of phylogenetic trees of S gene, complete genome, and N gene suggest the occurrence of potential recombination events (Fig. 1A and B).

SimPlot and Bootscan analyses of the five complete FRCoV genomes were performed to investigate the genetic variability in different parts of the genome and potential recombinations.[7] The FECV1(US) strain was used as the query and compared with four strains: FRCoV_NL_2010(NL), FSCV6(US), ferret063(Japan), and FRCoV4370(Japan). Higher genetic variability was observed in the S
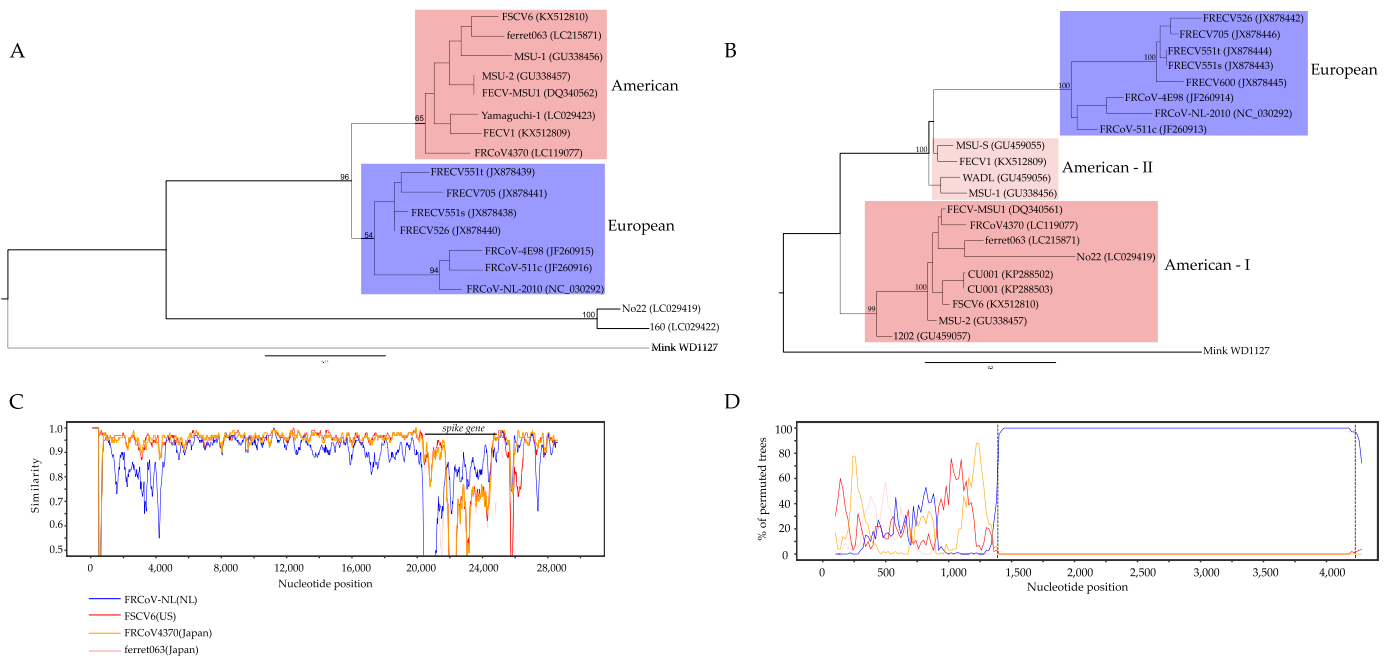
Fig. 1. Phylogenetic analyses for N gene (A), and S gene (B) of FRCoVs. FRCoVs from America and Europe are indicated by red and blue boxes, respectively. Two genetic groups identified for S gene of American FRCoVs are represented by American-I and American-II. Numbers at the nodes indicate bootstrap support evaluated by 500 replicates. Recombination analyses of S gene of FRCoVs (C and D). The FECV1 strain from America was used as the query and compared with four strains: FRCoV_NL_2010 (NL), FSCV6(US), ferret063(Japan), and FRCoV4370(Japan). (C) SimPlot shows the genetic distance between query sequence and each reference sequence in different parts of the genome. (D) Bootscan plot shows phylogenetic relationship between query sequence and reference sequences in different parts of the S gene. Potential region for recombination event is highlighted by dash lines.

Table 1

Comparison of putative transcription regulatory sequences (TRS) for ORF1ab, spike, membrane, and nucleocapsid genes between five ferret coronavirus (FRCoV) strains. The core TRS is indicated in bold. Accession number and origin for each strain: FRCoV-NL-2010 (NC_030292, the Netherlands), FECV1 (KX512809, the United States), FSCV6 (KX512810, the United States), Ferret063 (LC215871, Japan), and FRCoV4370 (LC119077, Japan).

Strain	ORF1ab	Spike	Membrane	Nucleocapsid
NL-2010	TCAA**CTAAAC**GAAA	GTTA**CTAAAC**TTTG	TCAA**CTAAAC**AAAATG	AGAA**CTAAAC**TTCTATTATG
FECV1		ATTA**CTAAAC**TTTG	TCAA**CTAAAC**AAAATG	AGAA**CTAAAC**TTCTATTATG
FSCV6		ATTA**CTAAAC**TTTG	TCAA**CTAAAC**AAAATG	AGAA**CTAAAC**TTTTATCATG
Ferret063	TCAA**CTAAAC**GAAA	ATTA**CTAAAC**TTTG	TCAA**CTAAAC**AAAATG	AGAA**CTAAAC**TTTTATCATG
4370	TCAA**CTAAAC**GAAA	ATTA**CTAAAC**TTTG	TCAA**CTAAAC**AAAATG	AGAA**CTAAAC**TTCTATCATG

gene, particularly at the 3′terminal end, compared to other parts of the genome (Fig. 1C). The average shared sequence identity was 91.0% for the complete genome, 93.1% for N gene, 95.0% for RdRp gene, and 73.6% for S gene, respectively. The 3′terminal end of S gene of FECV1(US) shared higher sequence identity with FRCoV_NL_2010(NL), despite the fact that FECV1(US) is more similar to FRCoVs from America in other parts of the genome. Bootscan analysis identified a potential recombination for the region between 1400 bp and 4260 bp in the S gene (Fig. 1D). Next, separate phylogenetic analyses were conducted for the recombination part and non-recombination part of the S gene. Phylogeny for the recombination part showed that American-II sub-group is closer to European lineage, whereas phylogeny for the non-recombination part showed that American-I and American-II sub-groups are both separated from European lineage (Fig. S3). Comparison of the putative transcription regulatory sequences (TRS) for these five strains showed identical core TRSs (Table 1). Taken together, these results suggest potential genetic recombination event at the 3′terminal end of S gene between FRCoVs from European and American lineages. However, due to relatively low sequence identity in the S

gene and small number of available sequences, other possibilities cannot be excluded.

This study focused on the S gene, and recombination could happen in other regions of the genome. Earlier studies have identified recombinations in the 3c and envelop genes.[8,9] The N and ORF 7b genes of two Japanese strains, No.22 and No.160, were completely different from those from other FRCoV strains.[9] The phylogeny of the N gene in this study supported the observation; phylogeny of the RdRp gene also showed that these two strains are distant from other strains (Fig. 1 and Fig. S2). The sequence identities in the RdRp gene between these two strains and other FRCoVs are relatively low (87.5%). While the source of these two Japanese strains are not clear, potential recombination, including recombination with other CoVs, could have contributed to the uniqueness of their genomes.

A critical question yet to be answered is the molecular basis for pathotype switch between less pathogenic FRECV and pathogenic FRSCV. Recombination in the S gene has been suggested to associate with the transmissibility and virulence of coronaviruses.[10] It is reasonable to propose that FRSCV may have evolved from FRECV

through recombination.[8,9] Although this study cannot establish a direct link between the detected recombination and change of pathogenicity, it is interesting to see that 3 out of 4 strains are FRSCV, including MSU-S, WADL, and MSU-1 (Fig. 1B). Future *in vivo* experiments are needed to clarify the precise biological implications of this recombination in S gene. One major limit of this study is the small number of FRCoV sequences available in the public databases. Considering the wide spread of FRCoV and extensive use of ferret as an animal model for influenza pathogenicity study, enhanced surveillance is required to monitor the spread and genetic diversity of FRCoV.

Declaration of Competing Interest

The author declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.01.016.

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Emergence of SARS-like coronavirus poses new challenge in China

Dear Editor,

Recently, the emergence of African swine fever virus in China has raised great concern in this journal.[1,2] The epidemic is not over yet, outbreak of a new SARS-like Coronavirus in Wuhan at the end of 2019 poses new public health challenges in China. Coronaviruses are a large family of viruses that cause respiratory illnesses. Although Coronaviruses (CoVs) have been known for decades, they did not raise great attention in human medicine until the outbreaks of Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS).[3] SARS-CoV first emerged in November 2002 in Guangdong province of Southern China and then rapidly spread to 29 countries and regions, infecting over 8000 individuals with a death toll of nearly 800.[3] Ten years after the SARS, MERS emerged in 2012, have caused 2494 human infections with 858 deaths (as of November 2019) and remains a disease of global, and particularly Middle Eastern, public health concern. In 2017, a novel HKU2-related bat coronavirus, swine acute diarrhea syndrome coronavirus (SADS-CoV), caused the death of 24,693 piglets,[4,5] raising further concern about these coronavirus. In December 2019, a new coronavirus (2019-nCoV), which is about 70% similar to SARS-Cov, was discovered in the central Chinese city of Wuhan, with 545 cases in 25 provinces being diagnosed (Fig. 1A, January 22, 2020). According to the World Health Organization (https://www.who.int), in addition to mainland China, this virus has also been detected in Japan, Thailand, Republic of Korea and the United States in travelers from Wuhan. The number of confirmed cases has been gradually increasing (Fig. 1B), which might be partially due to recent the establishment of relevant detection methods, but, at the same time, could signal that there could be a quick expansion of the epidemic.

The three basic elements required for an infectious disease epidemic are source of the infection, route of transmission, and susceptible hosts (humans). Eliminating the source of the infection and cutting off the transmission route are usually effective means to block the spread of an infectious diseases. The successful precedent of emergent CoV containment based on the elimination of the primary reservoir is SARS. Bats are suggested to be the reservoir hosts of SARS-CoVs.[6,7] However, without an intermediary host, bat derived CoVs cannot directly infect humans. The Carnivora–intermediate amplifying host (civets) of SARS-CoVs was found.[8] The quick control of the intermediate amplifying host by banning wild animal trade was the key factor in the effective control of SARS. Many of the 2019-nCoV infected people had either worked at or frequently visited a seafood market in Wuhan. Apart from fish, the market also sold other live wild animals – sparking concerns that the virus might have been transmitted from an animal to humans, just like SARS and MERS. The Wuhan CoVs cluster with SARS/SARS-like coronaviruses, and have about 70% overall genome sequence similarity. As this virus clusters with various bat Betacoronavirus, it is reasonable to deduce that bats are the native host for the 2019-nCov. However, its origin (source) remains unknown, that is, the animals that are the origin and amplifying hosts for this virus. This makes the elimination of this disease from the source difficult.

The control of the route of transmission is another effective means of epidemic control. Infections of healthcare workers and family clusters suggest that 2019-nCoV has the ability to spread from human to human. Although the seafood market has been closed, the number of confirmed cases continues to gradually

A

B
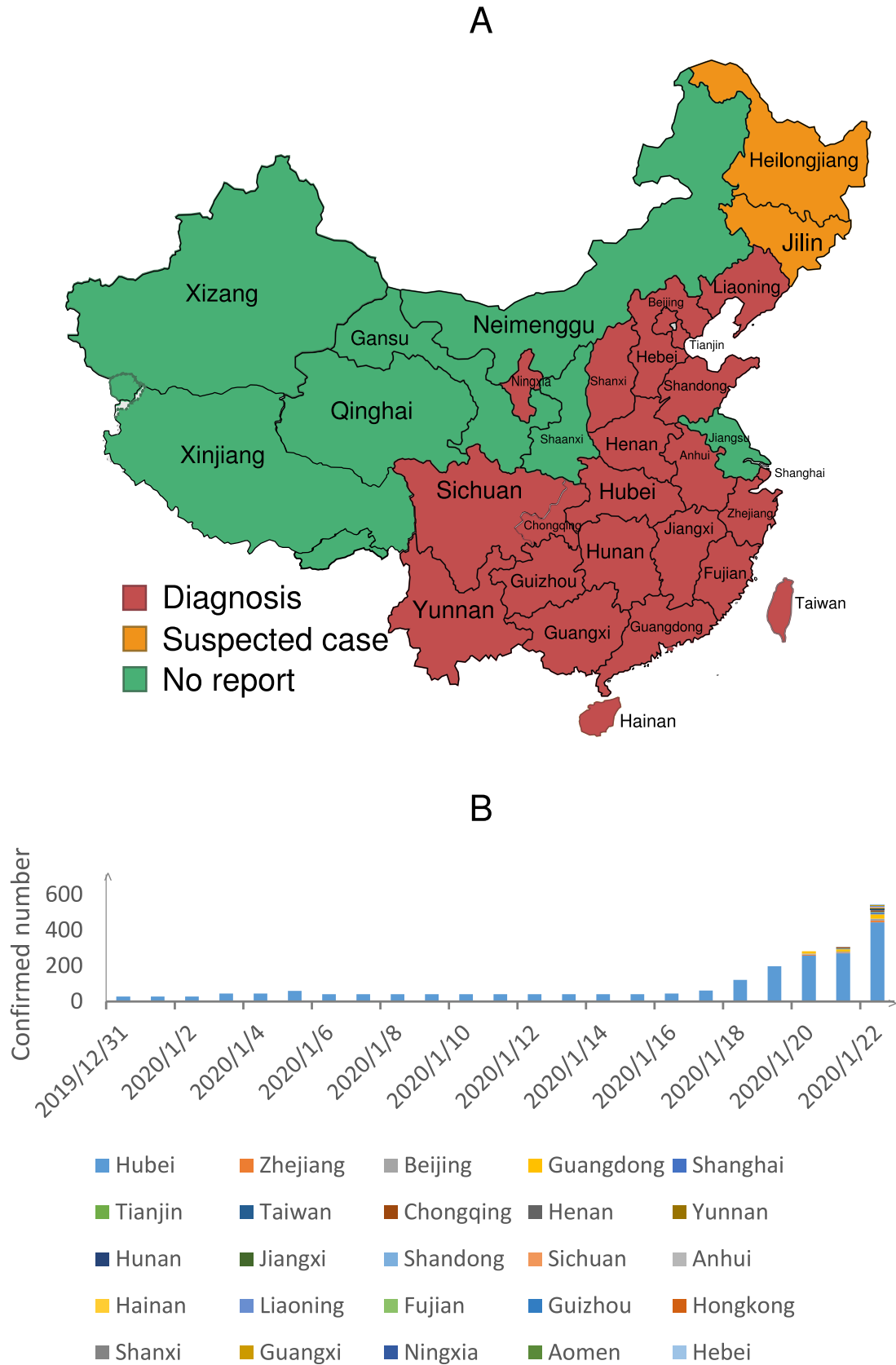
Fig. 1. The 2019-nCoV epidemic.
(A) Spread of 2019-nCoV in China. Provinces with 545 confirmed 2019-nCoV cases to date (1/22/2020 8:00 PM) are indicated in red (total 25). Provinces in yellow have suspected cases, while those in green have reported no cases. The epidemic is mainly distributed in central and southern China.
(B) Increasing number of cases. The number of confirmed cases by date are plotted, which shows a gradual increase over time.

increase ([Fig. 1](#)B). This further supports the conclusion that this virus can spread by human-to-human contact. Previous research has shown that coronaviruses usually have an ability to rapidly mutate.[9] We cannot exclude the possibility that some 2019-nCoVs will mutate to become "super-spreaders", as seen in SARS. Frequent disinfection of people-intensive places and animal trading markets should help stop the spread of the virus. Unfortunately, the outbreak has cast a shadow over the celebrations for the Lunar New Year, which falls on January 25. Millions of people in China travel over the course of the Spring Festival, both within the country and overseas. In addition, millions of college students (Wuhan has more than 1 million college students) will return to school after the winter vacation. This large-scale population migration could lead to further spread of this virus. Therefore, it is possible that this epidemic will be more serious after the Spring Festival. At present, the National Health Commission of China (http://www.nhc.gov.cn) has listed 2019-nCoV as Class B infectious disease and managed as Class A. Measures including the temperature monitoring of passengers at railway stations, airports, terminals and other transportation hubs have been carried out, which should slow the spread of the virus to a certain extent. However these disease control policies are far from enough, more strict methods to control population flow should be on the table.

It is increasingly recognized that a One Health approach at the human–animal–ecosystem interface is needed for effective investigation, prevention and control of any emerging zoonotic disease. In the context of emerging zoonoses, human and veterinary medicine must work together. These viruses emerge from animal trading markets, where veterinary workers need to be vigilant and concerned whether these viruses have the potential to spread between animals and the impact the livestock industry. Veterinary workers should also actively invest in epidemiological investigations to find the natural hosts of these viruses and develop corresponding detection methods. Considering that the original source of the 2019-nCoV is very likely related to a wild animal, additional strict laws to limit wildlife markets should be made, otherwise, more emerging zoonoses from wild animals will occurred in the near future.

Acknowledgments

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Getah virus: An increasing threat in China

Dear Editor,

Several recent studies in this journal have highlighted the threat posed by mosquito-borne viruses in China, such as Zika virus and dengue virus.[1-3] Getah virus (GETV) is also a mosquito-borne virus that is classified in the *Alphavirus* genus of the *Togaviridae* family. The genome of GETV is linear, positive-sense ssRNA, encoding a total of nine viral proteins (nsP1-nsP4, E1-E3, C, and 6 K). GETV was first isolated from *Culex* mosquitoes collected in Malaysia in 1955 and has since been found in mosquitoes in Asian countries surrounded by the Pacific Ocean (China, Japan, South Korea, Mongolia, Russia, and India) based on viral isolation and/or molecular epidemiological investigations.[4,5]

GETV infection has been found in multiple vertebrates, including humans, monkeys, birds, pigs, horses, and other mammals, and infection of these species is essential for the maintenance of GETV zoonotic transmission cycles in nature. However, GETV has long been regarded as pathogenic only in pigs and horses. GETV infection can cause fever, generalized rash, and leg edema in horses and is often associated with fetal death and reproductive disorders in pigs. Several disease outbreaks caused by GETV infection in pigs and horses have been reported in Japan and India.[6]

In China, the epidemic status of GETV has become increasingly problematic recently, posing a great threat to animal and public health. Initially, GETV was detected in several new mosquito species. GETV has been regarded as being primarily carried and spread by mosquitoes of the genera *Aedes* and *Culex*. In China, GETV was first isolated from wild *Culex* mosquitoes in Hainan province in 1964 and was recently detected in and/or isolated from other mosquito species, including *Aedex vexans, Armigeres obturbans, Armigeres subalbatus*, and *Anopheles sinensis*.[4,5,7-9] Unfortunately, these mosquito species are widely distributed in China with large populations. Currently, GETV has a wide distribution. Before 2006, GETV had been found in only six provinces in China.[8] However, the number of GETV-affected provinces dramatically
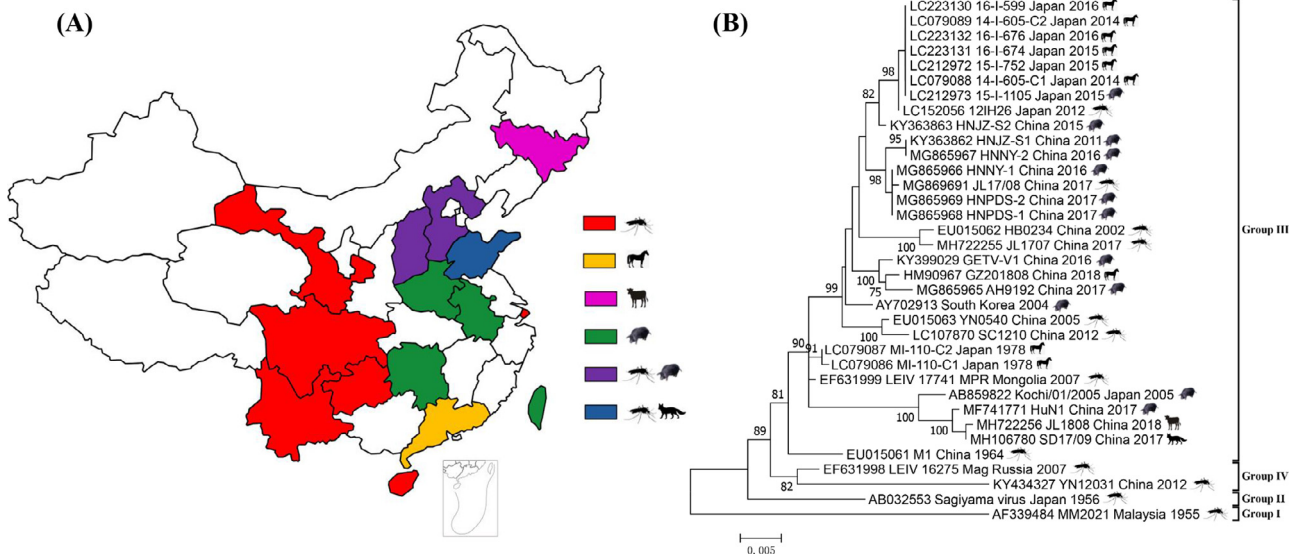
(A)

(B)

Fig. 1. Geographical distribution (A) and phylogenetic analysis (B) of GETV strains in China.
(A): The geographical distribution of GETV strains isolated from mosquitoes, horses, cattle, pigs, and foxes in China, as indicated by different colors.
(B): A maximum likelihood phylogenetic tree based on the E2 gene was constructed using the TN93+G method by using MEGA 7.0 software based on a bootstrap value of 1000 replicates. The GETV strain name, accession number, country of origin, animal of origin, and isolation year are indicated.

increased to fifteen in 2018 (Fig. 1).[4,5,7,9,10] In addition, more mammalian species were found to be infected with GETV. Direct molecular evidence supporting GETV infection in pigs in China was first obtained in Taiwan in 2002 and was obtained in five other provinces in Mainland China after 2011 (Fig. 1). In addition, horses infected with GETV in China were first identified in 2018.[10] Moreover, GETV infection in fox and cattle was reported in Northern China in 2019.[7,9] It should be noted that this was the first time that GETV was isolated from these two animal species in the world, which undoubtedly expanded the host range of this virus. Furthermore, GETV was determined to be pathogenic in several more vertebrate species. Neutralizing antibodies against GETV have been identified in human sera in China. The antibody titer in people with fever is significantly higher than that in healthy people, indicating that GETV infection is possibly associated with disease in humans. GETV infection in pigs and horses in China has been commonly associated with piglet death and fever, respectively. GETV infection could result in fever, anorexia, depression, neurological symptoms, and even death in foxes and has been associated with fever, anorexia, and depression in cattle. Moreover, the genetic complexity of GETV in China is increasing. Among the published GETV strains, the majority were isolated in China (Fig. 1). Most of the Chinese GETV strains obtained in the field were isolated after 2010. GETV strains worldwide can be classified into four groups, Groups I-IV, based on the sequence of the E2 gene (Fig. 1).[4] Nearly all GETV strains identified worldwide after the 1960s belong to Group III, except for one Chinese strain (YN12031, which was isolated from mosquitoes in 2012) and one Russian strain, which were classified as Group IV. In addition, some Chinese GETV strains in Group III show special genetic characteristics. For example, the HNJZ-S2 strain, which was isolated from pigs in 2015, has a closer relationship to Japanese GETV strains than other Chinese GETV strains. The JL1707 strain, which was isolated from mosquitoes in 2017, was clustered together with one mosquito-derived Chinese GETV strain in 2002 but showed a distant relationship with all six other Chinese GETV strains in 2017.

GETV is transmitted between individuals by mosquitoes, which serves as a vector, and the infected vertebrates act as amplifying hosts in GETV transmission cycles.[5] China has a vast territory with a wide variety of mosquito and vertebrate species. In China, the numbers of newly discovered vector and infected vertebrate species of GETV have increased rapidly in recent years. However, the threat posed by GETV has not yet attracted enough attention in China. To prevent and control GETV in China, it is necessary to take a series of related measures, such as increasing the popular knowledge of GETV, decreasing the density of mosquitoes, improving biosafety awareness, and strengthening epidemiological surveillance. In addition, vaccination is regarded as an effective strategy for GETV prevention and control. An inactive GETV vaccine has been developed in Japan to control the spread of this virus in horses (http://www.jp-nisseiken.co.jp/en/products/vaccine/index.html). However, the vaccine antigen composition is based on a GETV strain isolated in Japan in 1978 and cannot provide adequate protection against infection with currently circulating GETV strains, as shown by the GETV outbreaks in Japan in 2014.[6] Therefore, there is an urgent need to develop a GETV vaccine using a Chinese strain that is prevalent in the field as the antigen component to vaccinate susceptible animals to reduce the increasing threat of GETV to animal and public health in China.

Declaration of Competing Interest

None.

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Antibiotic prophylaxis in vaccinated eculizumab recipients who developed meningococcal disease ☆

Dear Editor,

We recently published a case series of typically commensal *Neisseria* spp. disease among eculizumab recipients.[1] Eculizumab is a terminal complement inhibitor indicated for treatment of paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, and certain patients with generalized myasthenia gravis or neuromyelitis optica spectrum disorder. [2] Due to complement inhibition, many different *Neisseria* spp. can cause invasive disease in eculizumab recipients[1,3,4] and eculizumab recipients are at an estimated 2000-fold increased risk of meningococcal disease (caused by *Neisseria meningitidis*). All eculizumab recipients should receive meningococcal vaccinations prior to therapy; however, eculizumab recipients may develop meningococcal disease or other *Neisseria* infections despite vaccine receipt.[4–6] For patients who cannot receive meningococcal vaccinations ≥2 weeks before starting eculizumab, U.S. eculizumab labeling recommends 2 weeks of antibiotic prophylaxis.[2] In July 2017, the Centers for Disease Control and Prevention (CDC) advised that prescribers could consider antibiotic prophylaxis in eculizumab recipients for the duration of eculizumab therapy.[4] There are no published studies evaluating the efficacy or safety of antibiotic prophylaxis in eculizumab recipients. Here, we report on potential risks and benefits of antibiotic prophylaxis for the prevention of meningococcal disease in a case series of vaccinated eculizumab recipients who developed meningococcal disease.

We searched for meningococcal disease cases in eculizumab recipients in the FDA Adverse Event Reporting System (FAERS) and the literature (PubMed, Embase) between March 2007 (U.S. approval date) and May 2017. The FAERS search retrieved 158 reports; no literature reports were identified that were not already identified in FAERS. Two FAERS reports were published in the literature after the FAERS search was conducted and were used to supplement the corresponding FAERS reports.[5,6] Of the 158 reports, 111 were excluded for the following reasons: lack of positive test for *N. meningitidis* ($n = 59$), indeterminate prophylaxis use/antibiotic not specified ($n = 20$), insufficient clinical course/timeline details ($n = 16$), duplicate ($n = 14$), or trimethoprim-sulfamethoxazole use (because a high proportion of *N. meningitidis* isolates are resistant to trimethoprim-sulfamethoxazole)[7,8] ($n = 2$).

Included patients received eculizumab within the three months preceding a diagnosis of meningococcal disease, defined as a report of a symptomatic patient with a positive culture or other confirmatory test for *N. meningitidis* from any body site. FAERS cases were matched to CDC meningococcal disease surveillance data from the National Notifiable Diseases Surveillance system, when possible, for confirmation of infection, serogroup,[1] and antibiotic susceptibility testing. Time to onset (TTO) was calculated from the date of first eculizumab dose (assumed to be the date of starting antibiotic prophylaxis) to the date of the patient's first meningococcal disease episode. For patients with multiple episodes of meningococcal disease, the calculations described below were performed using only the first meningococcal disease episode.

The series included 47 patients, of whom 15 were taking antibiotic prophylaxis at the time of meningococcal disease onset and 32 were not (Table 1). There were four fatalities due to meningococcal disease (all among patients not taking prophylaxis). All 47 patients reportedly received ≥1 dose of a meningococcal vaccine. Three of 47 patients had ≥1 episode of meningococcal disease

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Table 1
Characteristics of patients taking antibiotic prophylaxis and patients not taking antibiotic prophylaxis.

	Antibiotic Prophylaxis n=15**	No Antibiotic Prophylaxis n=32
Age* (years)		
Mean	21	31
Median	20	27
Range	7 - 40	5 - 83
Sex		
Male	5 (33%)	12 (38%)
Female	10 (67%)	20 (62%)
Reason for eculizumab use		
Paroxysmal nocturnal hemoglobinuria (PNH)	6 (40%)	20 (62%)
Atypical hemolytic uremic syndrome (aHUS)	7 (47%)	8 (25%)
Other	2≠ (13%)	4^ (13%)
Country		
U.S.	2 (13%)	13 (41%)
Non-U.S.	13 (87%)	19 (59%)
Number of patients who received at least one dose of meningococcal vaccine before first episode of meningococcal disease#		
Serogroup B vaccine (any brand%)	4	6
Serogroups A,C,W, and Y vaccine (any brand)	12	24
Time to onset to first episode of infection (days)	n=11 with data available	n=26 with data available
Mean	835	471
Median	835	333
Range	3 - 1873	8 - 2247
Serogroup of infection (per patient, first episode of meningococcal disease only), and vaccine history for patients with serogroup B, C, W, or Y disease		
B	5 — 1 of 5 patients vaccinated against B	10 — 0 of 10 patients vaccinated against B
C	1	3
W	1 — 4 of 5 patients vaccinated against infecting serogroup; 1¶ of 5 vaccine type unknown	2 — 6+ of 7 patients vaccinated against infecting serogroup
Y	3	2
E	0	1
X	0	1
Nongroupable	3	10
Not reported	2	3
Penicillin susceptibility testing, n (%)	n=6 isolates from 6 patients with data available	n=9 isolates from 8 patients with data available‖
Susceptible	1 (17%)	7 (78%)
Intermediate	3 (50%)	1 (11%)
Resistant	2 (33%)	1 (11%)

* Age at first episode of meningococcal disease, rounded down to nearest year

≠ n=2; membranoproliferative glomerulonephritis and C3 glomerulopathy

^ n=1 neuromyelitis optica; n=1 conflicting report in which PNH, aHUS, and renal transplant were each noted as a reason for eculizumab use; n=1 malignant hypertension; n=1 aHUS, antiphospholipid syndrome, and systemic lupus erythematous

All patients in the series reported a history of some type of meningococcal vaccination, but the serogroups covered by the vaccines (and vaccine brands) were not reported for every patient. The rows for serogroup B and serogroups A, C, W, and Y are not mutually exclusive; patients who received a serogroup B vaccine may also be counted in serogroup A, C, W, and Y vaccine row.

% Including serogroup B/C vaccine (non-U.S.)

¶ n=1 patient received meningococcal vaccine prior to developing meningococcal disease by Y serogroup, but the serogroups covered by the vaccine were not reported

+ Of the 6 patients with C, W, or Y disease: n=1 vaccinated patient received a serogroup B/C vaccine prior to developing serogroup C meningococcal disease; n=5 patients received ACWY vaccination prior to developing meningococcal disease by C, W, or Y serogroup. The 7th patient received serogroup C vaccine prior to serogroup W meningococcal disease

‖ n=1 patient had two episodes of meningococcal disease: 1st episode isolate penicillin susceptible, 2nd episode isolate penicillin resistant (both are counted)

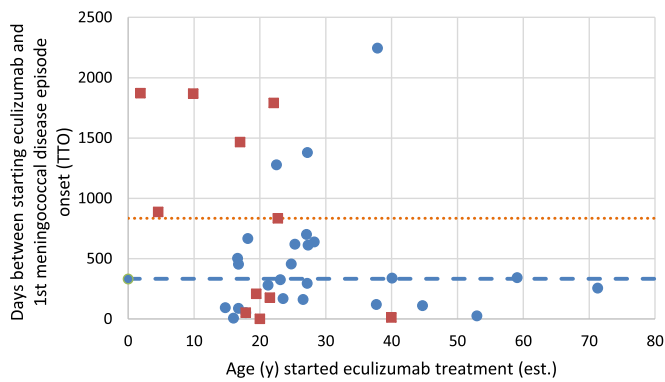** n=14 patients were taking penicillin, n=1 patient was taking amoxicillin

Fig. 1. Age (years) of patients at time of starting eculizumab therapy versus TTO of first meningococcal disease episode

The relationship between age when patients started treatment and TTO of first meningococcal disease episode, for prophylaxis recipients and non-prophylaxis recipients.

Key:

Red square = patients taking antibiotic prophylaxis with reported TTO ($n = 11$)
Blue circle = patients not taking antibiotic prophylaxis with reported TTO ($n = 26$)
Orange … dotted line = median TTO (835 days) for patients taking antibiotic prophylaxis with reported TTO ($n = 11$)
Blue - - dashed line = median TTO (333 days) for patients not taking antibiotic prophylaxis with reported TTO ($n = 26$)
Note, one patient in the "prophylaxis group" was 18 years of age with a TTO=53 days, and one patient in the "no prophylaxis group" was also 18 years of age with a TTO=53. These data points overlap on the figure.

(two patients were taking prophylaxis; one was not). TTO of first episode of meningococcal disease was reported for 11 of 15 patients taking prophylaxis and 26 of 32 not taking prophylaxis. Median TTO of first episode of meningococcal disease was 835 days in prophylaxis recipients versus 333 days in patients not taking prophylaxis. The range of TTO was large in both groups (3–1873 days in prophylaxis recipients vs. 8–2247 days in non-prophylaxis recipients). Among patients with susceptibility results available, penicillin non-susceptibility was reported more frequently in patients taking prophylaxis (5 of 6 isolates, 83%) than in patients not taking prophylaxis (2 of 9 isolates, 22%).

With the limited data available in both the FAERS reports and published reports, it is not possible to determine whether antibiotic prophylaxis is effective in preventing meningococcal disease in eculizumab recipients. However, in this descriptive analysis we observed a prolonged TTO of first meningococcal disease episode and a higher frequency of reduced penicillin susceptibility among prophylaxis users compared to non-prophylaxis users (all previously vaccinated). Although these results suggest that antibiotic prophylaxis may delay TTO, prescribers should interpret these findings conservatively, particularly given the wide range of TTO and the substantial differences in patient age and country of residence between prophylaxis recipients and non-prophylaxis recipients.

Prescribers must weigh the potential risks of antibiotic prophylaxis, such as adverse events and antibiotic resistance, against the potential benefits. When considering the benefit-risk balance of prophylaxis use, the more frequent reports of reduced penicillin susceptibility among prophylaxis users deserve comment. If this is a true relationship, colonization by meningococcal isolates with reduced penicillin susceptibility may have precluded penicillin from preventing meningococcal disease. Reduced penicillin susceptibility could be a consequence of selective antibiotic pressure. Further complicating use of prophylaxis, eculizumab recipients are also at risk for disseminated *Neisseria gonorrhoeae*,[3] an organism in which penicillin resistance is common.

The analysis has several limitations inherent to the data source. Cases were derived from spontaneous reports, which are sub-

ject to underreporting of outcomes, biased reporting, and variable quality. Confounding of the relationship between antibiotic prophylaxis and TTO is possible since patient characteristics, including age (Fig. 1) and country of residence, differed substantially between groups. Ascertainment of prophylaxis exposure was challenging and inconsistent prophylaxis use could reduce differences in TTO between groups. Other important data deficiencies include missing information on meningococcal antimicrobial susceptibility testing results, methods, and breakpoint criteria.

Overall, the data are inconclusive. However, we did observe a trend towards prolonged TTO among prophylaxis users but also towards increased penicillin non-susceptibility among prophylaxis recipients. Validation of these potential associations in a larger sample, with systematic ascertainment of antibiotic exposure, could further elucidate the potential impact of prophylaxis on development of meningococcal disease among eculizumab recipients. Healthcare professionals should remain vigilant for signs of meningococcal disease among eculizumab recipients, irrespective of the preventive measures in use.

Declaration of Competing Interest

The authors have no potential conflicts of interest to disclose.

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Detection of 20 respiratory viruses and bacteria by influenza-like illness surveillance in Beijing, China, 2016–2018

Dear Editor,

We read with interest the recent report by Lam and colleagues in this Journal, who compared global rates of respiratory viruses (REF)(1). We found that 20 respiratory pathogens circulated in Beijing, China, and influenza virus, human rhinovirus (hRV) and mycoplasma (MP) were the major pathogens. This information needs to be considered by clinicians when treating patients presenting with influenza-like illness (ILI).

Influenza viruses, other respiratory viruses and bacteria have been detected in patients with ILI (2–4). These respiratory viruses and bacteria, including seasonal humans influenza virus A (sFluA) and humans influenza virus B (sFluB), human coronavirus (hCoV-OC43,hCoV-NL63,hCoV-HKU1 and hCoV-229E), para-influenza virus (PIV 1–4), adenovirus (ADV), enterovirus (EV), hRV, respiratory syncytial virus (RSV), boca virus (BoV), human metapneumovirus (hMPV), chlamydia (CM) and MP are well recognized. Patients infected by these pathogens exhibit highly similar symptoms, rendering a clinical diagnosis unreliable and limiting aetiological and epidemiological studies.[2] Elucidating the epidemiological characteristics and regularity of ILI pathogens is of great significance in guiding clinical diagnoses and avoiding the abuse of antibiotics.

We collected 6327 throat swabs. Of the 6327 (female 2782 *vs.* male 3545) outpatients who sought treatment in 2016 to 2018, 1886 outpatients were confirmed as pathogen positive. In this study, we studied only ILI outpatients who were single pathogen positive. Overall, the most frequently detected agents were H3N2 (530/6327, 8.38%), H1N1 (285/6327, 4.50%), hRV (216/6327, 3.41%), and B-Yamagata (195/6327, 3.08%). (Fig. 1 and Table 1). In short, the positive rate of each year was basically the same as the total positive rate. The top three pathogens were mainly influenza viruses. Influenza and other pathogens were present for most of the 3 years in this study. However, the same influenza subtype did not persist as the dominant strain; rather, a mixture of high-intensity peaks of single subtypes and the co-circulation of types and subtypes at variable intensities occurred. (Fig. S1–3). This was consistent with other ILI surveillance data that demonstrated asynchronous peaks and the co-circulation of different pathogens.[5]

Of the 6327 ILI, the overall prevalence of influenza was 18.90% (1196/6327). Relatively low detection rates have even been reported in studies conducted in other geographical areas, such as Gansu Province in China,[6] in which a prevalence of 14.22% (501/59,791) was reported in a study conducted between 2010 and 2016. Nevertheless, other studies have reported relatively high detection rates in regions such as the Northern Hemisphere (2013–2014)[4] and Guangdong Province in China (2017–2018),[7] at 20.48% (1086/5303) and 28.33% (2137/7543), respectively. Regarding the pathogen spectrum of ILI in Beijing, the dominant strains in 2016–2018 were H3N2 and B-Victoria (2016), H3N2 and H1N1 (2017), H3N2 and B-Yamagata (2018), indicating that different dominant subtypes of influenza viruses in this region alternate. The discrepancies in the influenza detection rates among patients with ILI from different areas highlighted the geographical differences in virus burdens. However, these geographical differences in the detection rate may have been affected by several other factors, such as different technical approaches, sampling periods, study durations, or target populations (global population, paediatric population, etc.).[1,3]

A Pearson correlation analysis was performed considering the numbers of influenza-positive cases and non-influenza cases; the
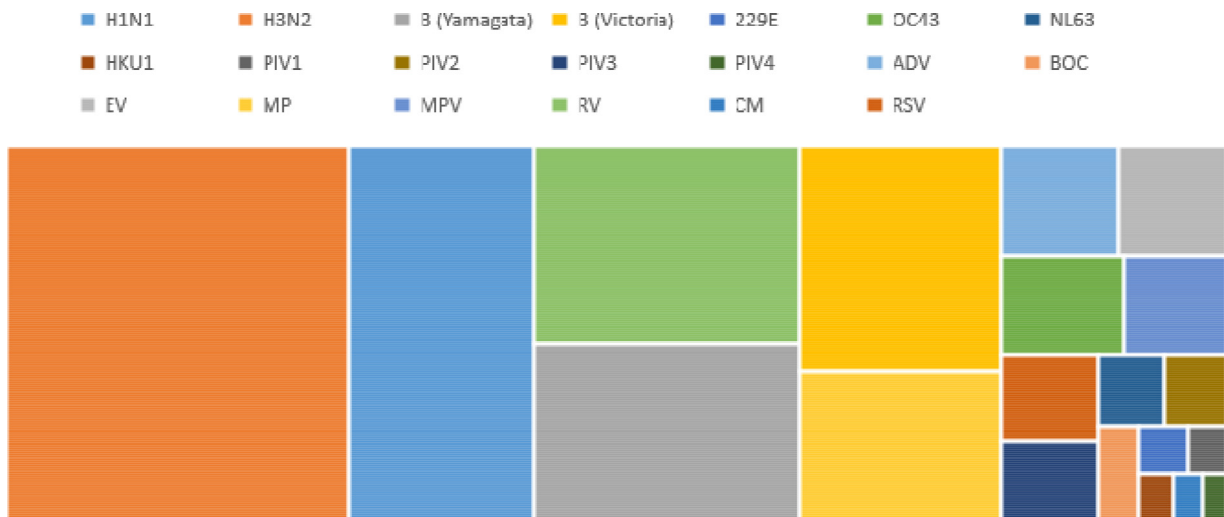

Fig. 1. Dendrogram of pathogen spectrum of ILI in Beijing from 2016 to 2018.

Table 1

Composition of ILI pathogen spectrum in Beijing, 2016–2018.

	H1N1 Positivity (%)	H3N2 Positivity (%)	B -Yamagata Positivity (%)	B -Victoria Positivity (%)	hRV Positivity (%)	BoV Positivity (%)	PIV-1 Positivity (%)	PIV-2 Positivity (%)	PIV-3 Positivity (%)	PIV-4 Positivity (%)	hCoV-229E Positivity (%)	hCoV-OC43 Positivity (%)	hCoV-NL63 Positivity (%)	hCoV- HKU1 Positivity (%)	EV Positivity (%)	hMPV Positivity (%)	RSV Positivity (%)	ADV Positivity (%)	CM Positivity (%)	MP Positivity (%)
Tatal	285(4.50)	530(8.38)	195(3.08)	186(2.94)	216(3.41)	16(0.25)	8(0.13)	18(0.28)	33(0.52)	5(0.08)	10(0.16)	49(0.77)	19(0.30)	7(0.11)	50(0.72)	42(0.66)	32(0.54)	52(0.82)	6(0.09)	127(2.01)
Age																				
0–4yrs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5–14yrs	1(2.33)	1(2.33)	1(2.33)	6(13.95)	2(4.65)	1(2.33)	0	0	1(2.33)	0	0	0	1(2.33)	0	0	1(2.33)	0	2(4.65)	0	0
15–24yrs	20(1.86)	85(7.92)	25(2.33)	38(3.54)	49(4.57)	3(0.28)	1(0.09)	6(0.56)	8(0.75)	0	5(0.47)	3(0.28)	5(0.47)	0	12(1.12)	1(0.09)	12(1.12)	7(0.65)	1(0.09)	20(1.86)
25–59yrs	221(5.11)	335(7.74)	138(3.19)	121(2.80)	141(3.26)	7(0.16)	5(0.12)	11(0.25)	16(0.37)	5(0.12)	4(0.09)	40(0.92)	6(0.14)	6(0.14)	34(0.79)	24(0.55)	16(0.37)	37(0.86)	5(0.12)	92(2.13)
60+yrs	43(4.87)	109(12.34)	31(3.51)	21(2.38)	24(2.72)	5(0.57)	2(0.23)	1(0.11)	8(0.91)	0	1(0.11)	6(0.68)	7(0.79)	1(0.11)	4(0.45)	16(1.81)	4(0.45)	6(0.68)	0	15(1.70)
χ^2	26.774	21.501	4.513	15.597	7.86	13.942	7.197	7.933	12.248	7.882	11.763[a]	5.133[a]	19.927[a]	7.343[a]	5.621[a]	25.875[a]	11.765[a]	8.842[a]	7.117[a]	2.915[a]
P-value	0.000[a]	0.000[a]	0.411[a]	0.050[a]	0.116[a]	0.016[a]	0.678[a]	0.283[a]	0.035[a]	0.504[a]	0.079	0.274	0.002	0.738	0.424	0	0.042	0.113	0.844	0.811
Gender																				
Female	167(6.00)	307(11.04)	123(4.42)	106(3.81)	119(4.28)	12(0.43)	6(0.22)	9(0.32)	18(0.65)	4(0.14)	8(0.29)	30(1.08)	10(0.36)	5(0.18)	25(0.90)	24(0.86)	19(0.68)	35(1.26)	3(0.11)	69(2.48)
Male	118(3.33)	223(6.29)	72(2.03)	80(2.26)	97(2.74)	4(0.11)	2(0.06)	9(0.25)	15(0.42)	1(0.03)	2(0.06)	19(0.54)	9(0.25)	2(0.06)	25(0.71)	18(0.51)	13(0.37)	17(0.48)	3(0.08)	58(1.64)
χ^2	25.915	45.72	29.792	13.184	11.229	6.269	3.131	0.266	1.506	2.637	5.278	5.967	0.58	2.145	0.744	2.978	3.098	11.591	0.089	5.647
P-value	0	0	0	0	0.001	0.012	0.077	0.606	0.22	1.104	0.022	0.015	0.446	0.413	0.388	0.084	0.075	0.001	0.766	0.017
Years																				
2016	55(2.66)	173(8.38)	27(1.31)	148(7.17)	88(4.26)	9(0.44)	1(0.05)	7(0.34)	3(0.15)	0	2(0.10)	29(1.40)	4(0.19)	2(0.10)	16(0.77)	15(0.73)	4(0.19)	30(1.45)	0	48(2.32)
2017	79(3.61)	277(12.65)	63(2.88)	11(0.50)	52(2.38)	5(0.23)	4(0.18)	8(0.37)	11(0.50)	3(0.14)	7(0.32)	11(0.50)	1(0.05)	3(0.14)	20(0.91)	15(0.69)	12(0.55)	7(0.32)	6(0.27)	33(1.51)
2018	151(7.28)	80(3.86)	105(5.07)	27(1.30)	76(3.67)	2(0.10)	3(0.14)	3(0.14)	19(0.92)	2(0.10)	1(0.05)	9(0.43)	14(0.68)	2(0.10)	14(0.68)	12(0.58)	16(0.77)	15(0.72)	0	46(2.22)
χ^2	57.587	107.306	49.367	194.483	12.057	4.802	1.599	2.15	11.884	2.631[a]	5.703	15.893	15.263	0.211	0.781	0.365	6.991	17.1	8.785[a]	4.308
P-value	0	0	0	0	0.002	0.091	0.449	0.341	0.003	0.379	0.058	0	0	0.9	0.677	0.833	0.03	0	0.003	0.116
Month																				
Jan	61(10.68)	114(19.96)	61(10.68)	26(4.55)	7(1.23)	1(0.18)	0	0	0	0	1(0.18)	1(0.18)	0	0	0	13(2.28)	7(1.23)	1(0.18)	0	5(0.88)
Feb	67(12.25)	76(13.89)	48(8.78)	52(9.51)	3(0.55)	0	0	1(0.18)	0	1(0.18)	0	0	0	1(0.18)	1(0.18)	5(0.91)	2(0.37)	3(0.55)	0	7(1.28)
Mar	60(12.24)	22(4.49)	16(3.27)	41(8.37)	11(2.24)	0	3(0.61)	0	0	0	0	2(0.41)	1(0.20)	0	1(0.20)	8(1.63)	2(0.41)	0	0	2(0.41)
Apr	28(4.90)	7(1.23)	9(1.58)	50(8.76)	35(6.13)	0	2(0.35)	2(0.35)	6(1.05)	1(0.18)	2(0.35)	9(1.58)	2(0.35)	3(0.53)	1(0.18)	9(1.58)	5(0.88)	2(0.35)	0	4(0.70)
May	4(0.77)	0	1(0.19)	15(2.89)	11(2.12)	7(1.35)	1(0.19)	8(1.54)	4(0.77)	0	1(0.19)	9(1.73)	1(0.19)	1(0.19)	7(1.35)	2(0.39)	0	0	0	3(0.58)
Jun	2(0.36)	3(0.53)	0	0	17(3.02)	2(0.36)	0	0	12(2.13)	0	0	10(1.78)	0	2(0.36)	4(0.71)	1(0.18)	1(0.18)	2(0.36)	6(1.07)	4(0.71)
Jul	1(0.18)	14(2.57)	0	0	34(6.25)	1(0.18)	3(0.55)	1(0.18)	4(0.74)	1(0.18)	0	5(0.92)	3(0.55)	0	6(1.10)	0	0	5(0.92)	0	4(0.74)
Aug	3(0.61)	56(11.38)	0	0	52(10.57)	0	0	2(0.41)	2(0.41)	0	0	4(0.81)	8(1.63)	0	11(2.24)	1(0.20)	3(0.61)	6(1.22)	0	14(2.85)
Sep	0	63(12.57)	1(0.20)	2(0.40)	16(3.19)	0	1(0.20)	0	2(0.40)	1(0.20)	1(0.20)	4(0.80)	3(0.60)	0	2(0.40)	1(0.20)	0	11(2.20)	0	27(5.39)
Oct	0	24(4.29)	0	0	15(2.68)	1(0.18)	0	2(0.36)	2(0.36)	1(0.18)	0	4(0.71)	1(0.18)	0	11(1.96)	0	7(1.25)	10(1.79)	0	31(5.54)
Nov	6(1.38)	37(8.53)	4(0.92)	0	12(2.76)	1(0.23)	1(0.23)	1(0.23)	1(0.23)	0	3(0.69)	1(0.23)	0	0	5(1.15)	0	3(0.69)	9(2.07)	0	17(3.92)
Dec	53(9.91)	114(21.31)	55(10.28)	0	3(0.56)	0	0	1(0.19)	1(0.19)	0	1(0.19)	0	0	0	1(0.19)	1(0.19)	2(0.37)	3(0.56)	0	9(1.68)
χ^2	54.668	113.198	343.292	314.972	144.03	19.865	10.32	20.174	32.867	6.9	9.463[a]	31.141[a]	23.843[a]	10.178[a]	37.559[a]	42.268[a]	21.338[a]	38.611[a]	19.020[a]	105.864[a]
P-value	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0	0.001[a]	0.120[a]	0.002[a]	0.000[a]	0.945[a]	0.297	0	0	0.118	0	0	0.007	0	0	0

[a] Fisher's exact test.

result showed that the former was not correlated with the latter (rs = −0.707, *P* < 0.05) (Fig. S4). Moreover, among the non-influenza respiratory pathogens, hRV and MP maintained high positivity levels, and the RSV infection rate increased annually (Table 1). The results of this study are similar to those of ILI in Ho Chi Minh city[5] and Zhuhai city.[2]

It was hRV that had a detection rate of 3.41% (216/6327) in this study; hRV had a great impact on persons under 60 years old and was a major viral pathogen of ILI during the study period (Table 1). In many regions of the world, there have been reports of outbreaks caused by hRV, such as the UK (2009–2017)[8] and Vietnam (2013–2015),[5] at 2.14% and 8.8%, respectively. Our study showed that hRV infection occurred predominantly in April, July, and August, with the majority of cases in the 5–24 years age group (Table 1). The virus was also highly associated with influenza and other pathogens. Respiratory symptoms caused by hRV are complicated, easily leading to a misdiagnosis and delayed treatment.[9] In the future, it is necessary to strengthen the monitoring of hRV in ILI patients, provide data support for the early warning and prevention of relevant epidemic situations, and avoid large-scale epidemics.

This study also found that MP accounted for a large proportion of the pathogen spectrum of ILI in Beijing, with a detection rate of 2.01%. MP is a common pathogen associated with human respiratory infections that can cause endemic or even global outbreaks among people of all ages.[10] However, there has been few studies on MP in routine surveillance at home and abroad, and the inclusion of these additional pathogens in ILI studies might greatly increase the positive detection frequency(2). Therefore, in an early epidemic of unexplained fever, it is necessary to be alert to the possibility of MP infection, especially when the epidemic occurs in a closed environment.

The strength of this study was that there were no previous reports on the detection of these 20 pathogens, especially MP and CM, in ILI patients during the influenza epidemic in Beijing. Our study showed that the data from this study people were important reference data, and that understanding the interactions between the different influenza subtypes and types and other respiratory pathogens is critical. In addition, we must improve prevention and management strategies for ILI.

These results demonstrate that a wide range of respiratory pathogens are circulating in Beijing city and that H3N2, H1N1, B-Yamagata, B-Victoria, hRV and MP are the major pathogens. It is recommended that the trend of pathogen spectrum changes in patients with ILI in the region should be continuously monitored. At the same time, the health department should also strengthen the analysis and utilization of monitoring data and track the activity level and variation in influenza virus rates to address fever outbreaks in a timely manner.

Declaration of Competing Interest

None.

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.11.014.

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Table 1
Characteristic of included studies.

Author/year	Country	Study sites	Mean age, year	No of PMX-HP patients	No of standard therapy	Study populations	Site of infection	Timing of initiating PMX-HP	Duration, no of secession (h)
Nakamura, 1999	Japan	Two centers	53.8	30	20	Septic shock	Respiratory system, urinary tract, bile tract, wound, intra-abdomen, peritonitis	6th day	2 h, twice
Nemoto, 2001	Japan	Single center	61.9	54	44	Severe sepsis/septic shock	Respiratory system, urinary tract, intra-abdomen, operation site, unknown	1st day	4 h, once or twice
Suzuki, 2002	Japan	Single center	64.5	24	24	Septic shock	NR	1st day	4 h, twice
Vincent, 2005	Belgium, UK, Germany, Netherlands, Spain, Japan	Multicenter	57.7	17	18	Severe sepsis/septic shock	Abdominal sepsis, surgical	1st day	2 h, once
Cantaluppi, 2008	Italy	Two center	60	8	8	Gram-negative sepsis	NR	1st day	2 h, twice
Cruz, 2009	Italy	Multicenter	63.8	34	30	Severe sepsis/septic shock	Abdominal sepsis, surgical	1st day	2 h, twice
Payen, 2015	France	Multicenter	69.8	119	113	Septic shock	Abdominal sepsis, surgical	1st day	2 h, twice
Dellinger, 2018	United State, Canada	Multicenter	59.8	224	226	Septic shock	Intra-abdominal, respiratory tract, urinary tract, dermatologic, cardiovascular, neurological	1st day	2 h, twice

PMX-HP, polymyxin B hemoperfusion; NR, not reported.

Effect of polymyxin B hemoperfusion on the outcome of patients with sepsis and septic shock

Dear Editor,

We read with great interest the report of the evaluating the use of apheresis for severe falciparum malaria, loiasis or babesiosis in a systemic review.[1] In this study,[1] Odedra et al. demonstrated that suggests, that apheresis may be a useful adjunct in the treatment of babesiosis, and loiasis. In addition to these uncommon infectious diseases, we are much more concerning the efficacy of another modality - polymyxin B hemoperfusion (PMX-HP), which can reduce blood endotoxin levels in sepsis, on the clinical outcome of patients with sepsis and septic shock. Therefore, we conducted a meta-analysis to investigate the clinical efficacy of PMX-HP on the mortality of patients with sepsis and septic shock.

From the literature review using Pubmed database, eight randomized clinical studies[2–9] compared the use of PMX-HP with standard therapy on the mortality of patients with sepsis and septic shock were identified. Table 1 summarized the characteristics of these included eight randomized trials.[2–9] Two studies[4,9] were multinational study and Japan was the most common country as study site. Six trials were multicenter studies and two trials were single center study. In addition to Nakamura et al.'s study,[5] all the other study initiated PMX-HP from the first day. Finally, the duration and the uses of PMX-HP varied according to each study design.

Overall, a total 993 patients were enrolled. Five hundred and ten and 483 patients were assigned to PMX-HP group and standard

therapy group, respectively. Those received PMX-HP and standard therapy had 28-day mortality rate of 36.3% (185/509) and 40.3% (195/483), respectively. No significant difference was observed between these two groups (Risk Ratio 0.76, 95% CI, 0.56–1.05, $I^2 = 69\%$). In subgroup analysis of two studies which performed PMX-HP for four hours per session,[6,8] the patients receiving PMX-HP had lower mortality rate with 48.7% (38/78) than standard therapy with 83.8% (57/68) (Risk Ratio 0.51, 95% CI, 0.25–1.05, $I^2 = 73\%$), but the difference did not reach statistical significance. Other six studies[2–5,7,9] evaluating the effect of PMX-HP for two hours per secession also showed that there was no significant difference in mortality among those received PMX-HP and those received therapy (Risk Ratio 0.90, 95% CI, 0.65–1.25, $I^2 = 51\%$).

In conclusion, based on the findings of this meta-analysis, it indicated that the survival benefit does not justify the routine use of PMX-HP to treat patients with sepsis and septic shock.

Declaration of Competing Interest

None.

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African swine fever virus in Asia: Its rapid spread and potential threat to unaffected countries

Dear Editor,

Recently, several reports in the *Journal of Infection* have highlighted the problem of African swine fever (ASF).[1,2] ASF is a highly contagious hemorrhagic disease of domestic pigs with a high morbidity and mortality. However, there are currently no effective vaccines to prevent and control this disease. The causative agent of ASF is ASF virus (ASFV), which was described and isolated for the first time in Kenya in 1921.[3] ASFV is endemic in most countries in sub-Saharan Africa and Sardinia. After 1957, this virus spread into European and American countries, and recently, it spread to Asia.

In Asia, except for Russia, no countries had experienced ASF outbreaks before 2018. In August 2018, China reported its first ASF outbreak.[4] In one year, ASFV quickly spread into all of the provinces in Mainland China. At the time we submitted this manuscript (November 12, 2019), a total of 164 ASF outbreaks in China had been recorded by OIE (Fig. 1) (https://www.oie.int/en/animal-health-in-the-world/information-on-aquatic-and-terrestrial-animal-diseases/african-swine-fever/reports-on-asf/). In 2019, after Mongolia reported its first outbreak in January, ASF rapidly spread into nine other Asian countries over the next eight months, including Vietnam (February), Cambodia (March), North Korea (May), Laos (June), the Philippines (July), Myanmar (August), Russia (August), South Korea (September), and Timor-Leste (September). As of now, a total of 6647 ASF outbreaks affecting 4,306,568 animals have been reported in Asia, resulting in great economic losses (Fig. 1). Moreover, the emergence and rapid spread of ASFV in Asia also poses a threat to unaffected countries.

ASF can be transmitted directly through contact between sick and healthy pigs or indirectly through untreated swill or other feed products. Asia raises a large number of pigs, and pork is the main meat source for local people. Among Asian countries, China is responsible for about half of the global pig population. Therefore, Asia has a high pig density. It should be noted that backyard and small-scale farms with weak biosecurity systems comprise a very large proportion of pig herds in Asian countries with a developing swine industry, such as in the as of yet ASF-unaffected Asian countries Indonesia, India, and Malaysia. ASFV is stable and infectious for a long period of time in blood, feces, tissue, and other viral contaminated products. However, it is common to feed pigs untreated swill in Asia. Therefore, food is also a potential source for ASF spread. Shortly after the first ASF outbreak in China, ASFV was found in dried pig blood used in pig feed, which is an important protein source for pigs in China. The developing swine industry with a high pig density but low biosecurity systems in Asia provides more opportunity for contact between healthy pigs and ASFV-affected pigs/ contaminants and may contribute to the spread of ASF in Asia in the past/future.

Luggage/pork products/waste from aircrafts/vessels/passengers from ASF-affected countries represent another important route for ASF spread. Japan and South Korea reported that ASFV was detected in pork products from China in April and August 2019, respectively. (https://www.nippon.com/en/news/yjj2019040200923/infectious-african-swine-fever-virus-found-in-japan-for-1st-time.html). With the development of the global economy as a whole, international economic cooperation and exchange are increasingly frequent. Countries with a developing/developed pig industry should implement enhanced national sanitary measures to monitor quarantine inspections of travelers from ASF-affected Asian countries.

Soft ticks of the genus *Ornithodoros* are considered to be reservoirs of ASFV and are widely distributed in Asia.[5,6] Several ASFV epidemiological cycles involving these ticks have been identified.[7] Surprisingly, a new ASFV variant strain was recently identified in hard ticks (*Dermacentor*) collected from sheep and bovines in China, expanding the vectors and hosts for ASFV.[8]

Wild boar is also susceptible to ASFV and plays an important role in ASF persistence in endemic areas or in sporadic outbreaks. Having no restricted movement area, wild boar is regarded as a source for further the geographic expansion of ASF.[9] In November 2018, ASFV was found in a dead wild boar at the border between China and North Korea.[10] Although there have been no further reports of ASFV in wild boar in Asia since then, considering wide geographical distribution of wild boar in both ASF-affected and -unaffected Asian countries, its potential role in ASF spread into unaffected counties should not be underestimated.

It should be noted that the latest ASF outbreak in Asia occurred in Timor-Leste (Fig. 1). Timor-Leste has a long geographical distance from the nearest ASF-affected Asian country, the Philippines ($\sim$ 2628 km), and is closer to Australia ($\sim$ 588 km). It is still unclear how ASFV spread into this country. However, the ASF
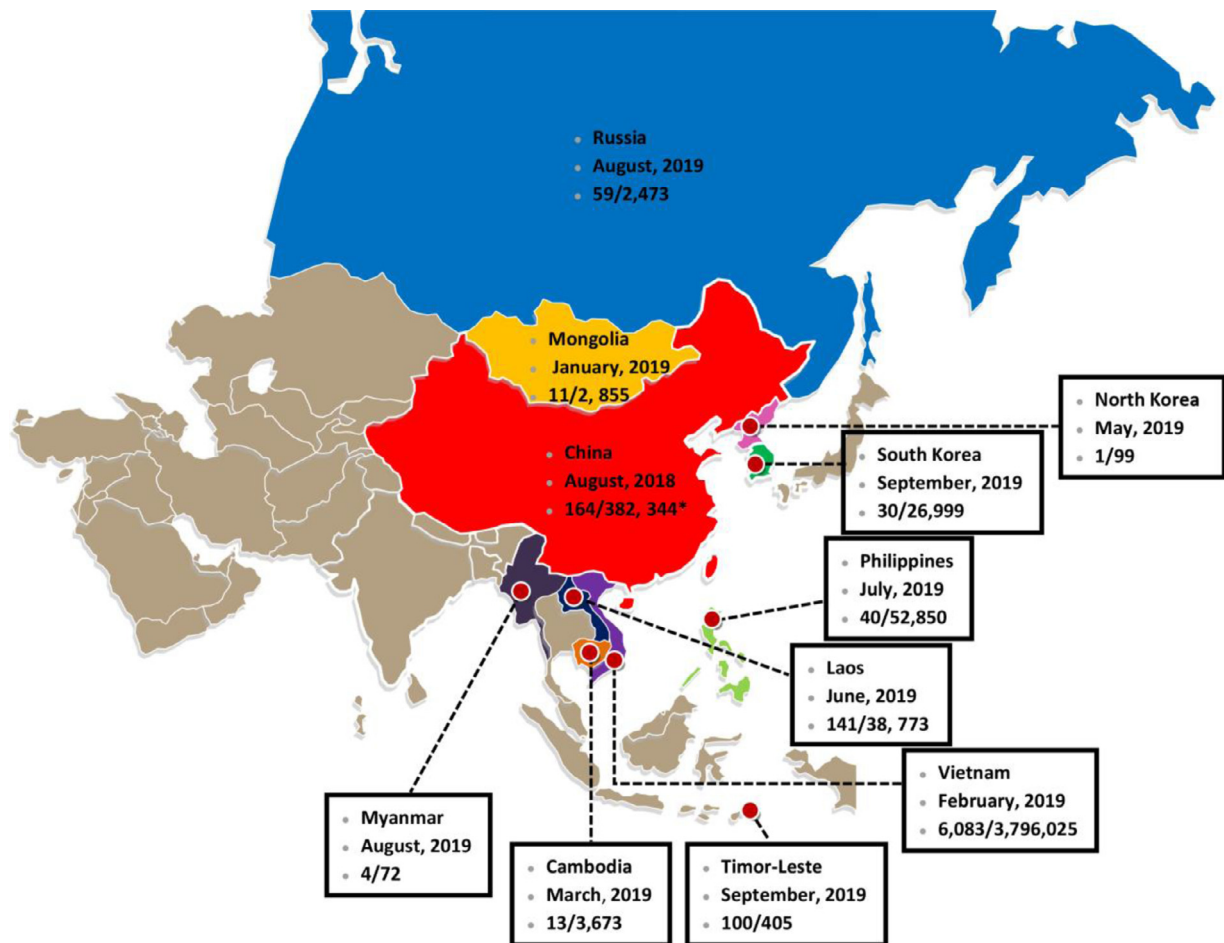
Fig. 1. ASF outbreaks in Asia since August 2018.
The ASF-affected country name and the time when the first outbreak of ASF was determined in that country are indicated. "∗" demonstrates the total ASF outbreaks / total animal losses in that country. For Russia, only its geographical area within Asia is shown.

outbreak in Timor-Leste is undoubtedly a great threat to pigs in Australia.

In summary, multiple factors are probably responsible for rapid spread of ASFV in Asia. To control the continuous spread of ASFV in Asia and reduce its potential threat to currently unaffected countries, there is a need for the joint updating of national policies and increasing international cooperation between different countries worldwide, such as increasing biosafety awareness, developing a modern pig industry, reducing the density of backyard pigs, strengthening the biosafety management of travelers and their luggage, and developing strategies for controlling related ticks and wild boar.

Declaration of Competing Interest

None.

Acknowledgments

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Rare homologous recombination in H3N2 avian influenza A viruses

Dear Editor,

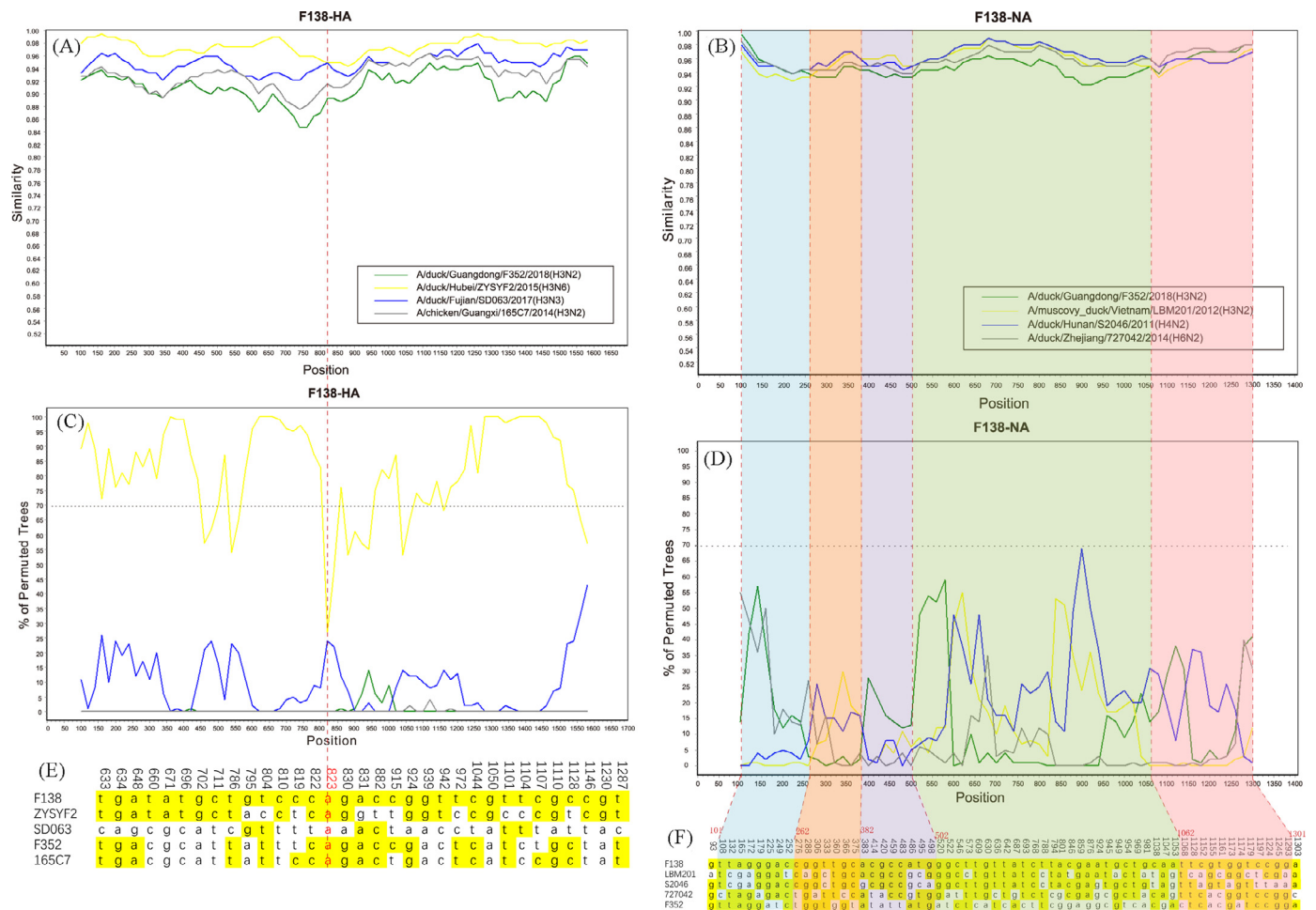Recently, Hu and colleagues declared in this journal that they had isolated two H3N2 avian influenza viruses (AIVs) that were derived from recombination events.[1] AIVs pose great challenges for disease control due to their rapid evolution. Their viral genomes are comprised of eight negative-strand RNA segments, and the lack of a proofreading mechanism during RNA replication results in a high frequency of point mutations. In addition to generating genetic diversity by rapid mutation, if multiple AIV strains coinfect a single cell, then the eight segments of the AIV genome can reassort and yield progeny virions with novel combinations of segments, a process termed reassortment. This is a very frequent process. In addition, homologous recombination is an important evolutionary mechanism that drives the formation of genetic variation for viruses that allows them to overcome selective pressures and adapt to new environments and hosts.[2,3] However, since AIV RNA is always encapsidated by a ribonucleoprotein complex, recombination in these viruses is very rare,[4–7] with some of the previously described putative recombination events proving to be false-positive signals.[8] Thus, the two H3N2 recombination events suggested by the Hu et al. study[1] are unexpected.

To examine these potential recombination events in more detail, we collected all available complete avian H3-HA and N2-NA AIV sequences from the public NCBI (https://www.ncbi.nlm.nih.gov), and GISAID (https://www.gisaid.org) databases. Redundant sequences and laboratory strains were removed. Finally, 2270 and 2883 unique H3-HA and N2-NA, respectively, sequences were used for our recombination analyses. A number of statistical methods have been developed to detect recombination in sequences, with each of the different methods having distinct performance characteristics and efficiencies.[9] Therefore, we performed recombination using the RDP4 program for the 3seq, bootscan, chimaera, genecov, lard, maxchi, rdp and siscan detection methods.[10] To avoid false positives, we recorded a recombination event only if it was detected to have a significant signal by at least three different methods. Using this approach, we detected three H3-HA sequences and seven N2-NA sequences that had strong signals for recombination (Table 1), however, these sequences did not include the two H3N2 viruses (A/duck/Guangdong/F138/2017 (H3N2) and

Table 1
Summary of recombination events in H3-HA and N2-NA segments identified by RDP4.

Gene	Recombinant strains	Parental sequences		Breakpoint position		P values for the seven detection methods in RDP4						
		Major parent	Minor parent	Beginning	Ending	Rdp	Geneconv	Boot scan	Maxchi	Chimaera	Siscan	3seq
HA	A/duck/Jiangsu/J3602/2014 (H3)	A/duck/Jiangsu/J3722/2014 (H3)	A/duck/Jiangsu/12.18_NJLH1266-P/2014(Mixed H3N2)	137	1236	<0.001	/	/	<0.001	<0.001	<0.001	<0.001
	A/duck/Jiangsu/J3722/2014 (H3)	A/pintail/Chany/198/2016 (H3N8)	Unknown	1242	1376	<0.001	<0.001	/	0.04	0.02	/	0.003
	A/duck/Vietnam/HU5-481/2016 (H3N8)	A/duck/Jiangsu/J2202/2014 (H3)	Unknown	53	1228	/	/	/	0.02	0.04	<0.001	0.001
NA	A/duck/Shantou/14665/2006 (H6N2)	A/Duck/Hubei/S4170/2008 (H6N2)	A/chicken/Ganzhou/GZ43/2016 (H3N2)	760	1407	<0.001	/	/	<0.001	<0.001	<0.001	<0.001
	A/Duck/Fujian/S4081/2008 (H6N2)	A/Duck/Hubei/S1366/2009 (H6N2)	A/goose/China/GX1167/2011 (H6N2)	770	1150	/	<0.001	<0.001	<0.001	/	/	<0.001
	A/duck/Sichuan/04.08_CDLQ0441-0/2015 (H4N2)	A/duck/Sichuan/04.08_CDLQ044-0/2015(Mixed H4N2)	Unknown	826	1224	/	/	/	<0.001	<0.001	/	<0.001
	A/chicken/Egypt/D4692A/2012(H9N2)92A/2012 (H9N2)	A/chicken/Egypt/D7108E/ 2013 (H9N2)	A/chicken/Egypt/NLQP194V-AR756/2013 (H9N2)	426	1407	/	/	/	<0.001	/	<0.001	<0.001
	A/mallard/Finland/13977/2010 (H9N2)	A/mallard/Finland/13384/ 2010 (H9N2)	A/mallard_duck/Netherlands/18/ 2009 (H6N2)	428	1104	/	/	/	0.003	/	<0.001	<0.001
	A/duck/Zhejiang/6DK19/2013 (H5N2)	A/duck/Zhejiang/6D4/2013(H3N2)	A/Taiwan/DV518/2006 (H5N2)	26	296	/	0.01	<0.001	<0.001	0.001	0.007	<0.001
	A/goose/China/Y114/2015 (H6N2)	A/goose/Guangxi/158/2013 (H6N2)	Unknown	1060	1407	/	/	/	<0.001	0.04	<0.001	0.02

Fig. 1. Recombination analyses of the F138 H3N2 AIV using SimPlot. (A) Similarity plotting analysis of the HA gene. (B) Similarity plotting analysis of the NA gene. (C) Bootscan analysis of the HA gene. (D) Bootscan analysis of the NA gene. (E) Variable sites in the F138 HA gene. (F) Variable sites in the F138 NA gene. The Similarity plotting analyses used a sliding window of 200 bases and a step size of 20 bases. The y axis shows the percent similarity between the selected AIV sequence and the query sequence. Bootscan analyses with the HA and NA genes of F138 used as query sequences. The y axis shows the percentage of permuted tree where the selected AIV strain clusters with the query sequence.

A/duck/Guangdong/F352/2018 (H3N2)) that were suggested to have recombination signals by Hu et al.[1]

To determine whether our strict criteria lead to the conflict with the result of Hu et al.,[1] we reanalyzed the data used by Hu et al. for potential recombination signals with the SimPlot program[11] used in their study. Our SimPlot graphs, which are the same as those in Hu et al.,[1] clearly show a high degree of similarity within the HA and NA sequences (Figs. 1A, B and 2A, B). We further used bootscan analyses to control the false positive signals. Signals of greater than 70% of the observed permuted trees indicate potential recombination events. However, the bootscan analyses demonstrate that there are no significant signals of recombination in these sequences (Figs. 1C, D and 2C, D). This suggests that Hu et al.[1] did not use a bootscan analysis to avoid detecting false positive signals, and thus reached an incorrect conclusion that F352 and F138 H3N2 AIVs were derived from recombination.[1] Examination of the variable sites in the sequences of the HA and NA genes of F138 and F352 (Figs. 1E, F and 2E, F) further suggest that the recombination regions by Hu et al.[1] have only 1–12 variable sites. AIVs have very high mutation rate. Random mutations can cause false positive signals.

In conclusion, we described an approach for the detection of recombination breakpoints in H3N2 AIVs and show that homologous recombination is very rare. When bioinformatic analyses are used to detect the presence of recombination, an assessment of the non-randomness of the signals is needed, and multiple methods must be used to avoid false positive results.

Declaration of Competing Interest

The authors declare not conflict of interest.

Acknowledgments

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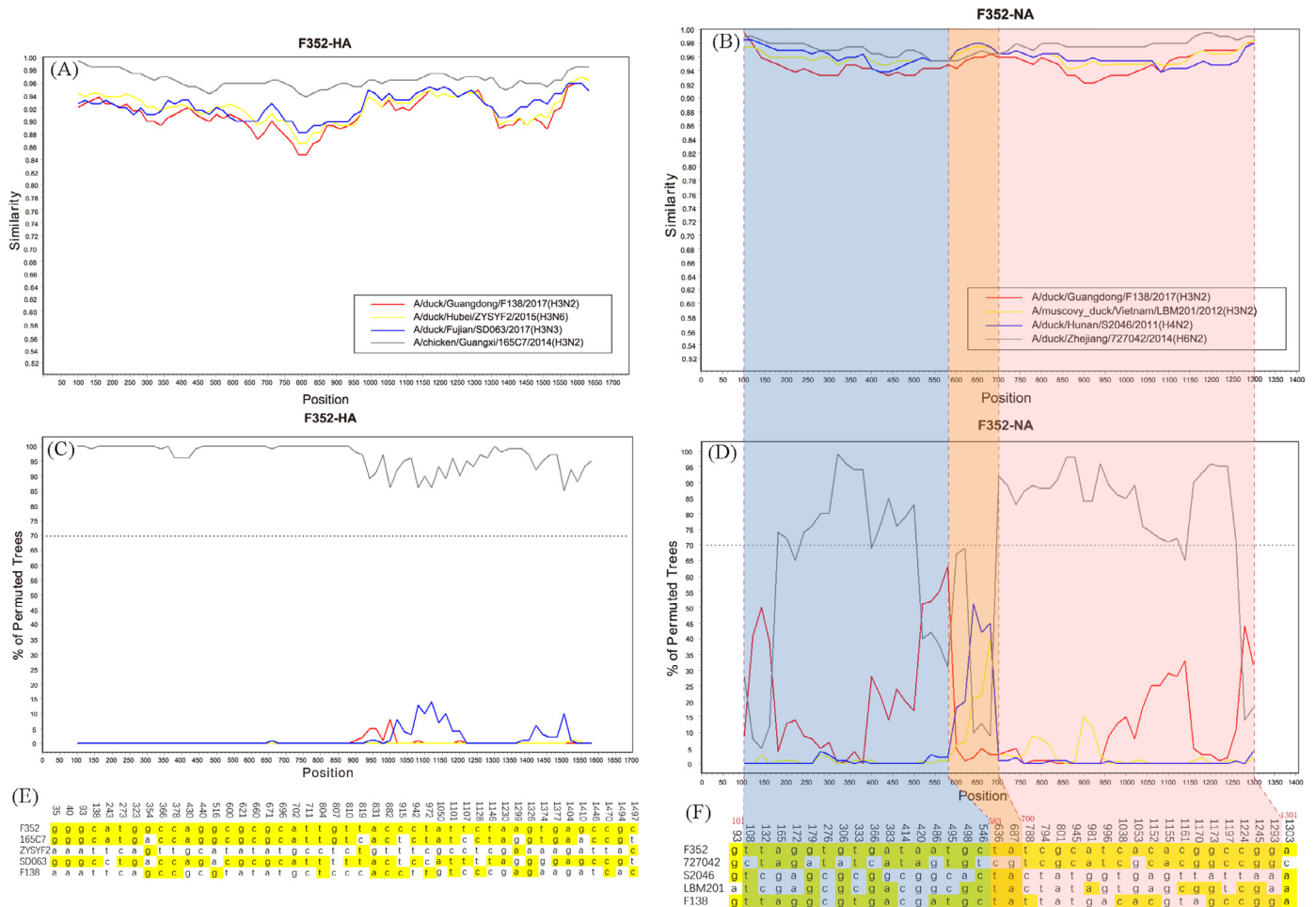
Fig. 2. Recombination analyses of the F352 H3N2 AIV using SimPlot. (A) Similarity plotting analysis of the HA gene. (B) Similarity plotting analysis of the NA gene. (C) Bootscan analysis of the HA gene. (D) Bootscan analysis of the NA gene. (E) Variable sites in the F352 HA gene. (F) Variable sites in the F352 NA gene.

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Table 1
Results of Xpert MTB/Ultra assay of the clinical samples.

Sample type		Number (%)	Number of positives (%)
Respiratory specimens	Sputum	148 (21,9)	13 (1,9)
	Bronchoalvelaor lavage	326 (48,3)	16 (2,3)
	Deep tracheal aspiration	10 (1,5)	
	Endotracheal aspiration	13 (1,9)	
	Gastric aspiration	69 (10,2)	
Extrapulmonary specimens	Cerobrospinal fluid	48 (7,1)	2 (0,3)
	Urine	61 (9,0)	2 (0,3)
Overall		675 (100,0)	33 (4,9)

Xpert MTB/Ultra assay: Handle with care

Dear Editor,

Dear editor, diagnostic performance of the Xpert MTB/RIF Ultra (Xpert Ultra) in comparison to Xpert MTB/RIF (Xpert) assay for the detection of paediatric pulmonary tuberculosis has been documented in this journal.[1]

End tuberculosis (TB) strategy aims to achieve 90% reduction in incidence and 95% reduction in mortality by 2035; obviously sensitive, rapid, accessible diagnostics is the most important factor to overcome TB which is one of the oldest and deadliest diseases of the mankind.[2]

Sputum smear microscopy is inexpensive, easy to perform and still the primary method for diagnosis of TB although maximum sensitivity has been found around 60% under optimal conditions.[3] Cultivation and antimicrobial susceptibility are still considered as gold standard, however due to lack of access to mycobacteriology laboratory facilities point of care tests are required for early diagnosis and to prevent dissemination of drug resistance strains all over the world.

Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is the most commonly used point-of-care assay for tuberculosis (TB) that was endorsed by WHO in December 2010. In 2014, this recommendation is expanded for use in all patients. Since the end of March 2017, WHO has recommended the replacement of Xpert by Ultra (Ultra, Cepheid, Sunnyvale, California). This advanced version has better TB detection capabilities and more definitive identification of rifampicin resistance especially important in problematic cases such as HIV coinfection, pediatric patients and extra pulmonary TB cases.[4] That increased sensitivity may predispose to false-positive results due to sample cross contamination, particularly in laboratories with heavy work load.[4,5]

We have replaced Xpert MTB/RIF assay to Ultra assay in December in our hospital that is localized in Istanbul, Turkey where 17 million inhabitants live. Incidence of tuberculosis (per 100,000 people) in Turkey was reported at 18 in 2015. We have processed 675 clinical materials from 515 patients by smear microscopy and culture and by Xpert Ultra assay between December 2018-May 2019. Specimens were first digested with N-acetyl-Lcysteine and sodium hydroxide and concentrated using standard methods. Smear microscopy was done using Ziehl-Neelsen and auramine-rhodamine staining . 0·5 mL of the resuspended pellet was inoculated into liquid culture using mycobacteria growth indicator tube (MGIT) with a BACTEC 960 instrument (BD Microbiology Systems, Sparks, MD, USA), and 0·2 mL was inoculated on Löwenstein-Jensen medium. Cultures positive for growth of acid-fast bacilli underwent confirmation of *M. tuberculosis* complex by MPT64/MPB64 antigen detection. Xpert Ultra assay was done by adding sample reagent to the first collected sputum specimen in a 2:1 dilution, and 2·0 mL of the resulting mixture was added to Xpert Ultra cartridge.

Respiratory specimens (sputum, brochoalveolar lavage, deep tracheal aspiration, gastric aspiration) was consisted 83.9% of the specimens where as 16.1% specimens were CSF and urine (Table 1). 217 (42, 1%) of the patients were women and mean age was 46, 33 years (1 month, 94 years of age)

Although 33 (4.9%) samples were PCR positive, we could not isolate *M. tuberculosis* from 11 samples. When repeated samples are excluded we analyzed 26 patients. Six patients (3 trace positive and 3 very low positive) were from bronchoscopy unit and we realized that positive results were detected following bronchoscopy of high positive patient. All of the patients were smear negative and clinician who performed the procedure was very anxious and wanted to know if they are false positive or not. We convinced her to wait for the results and we screened the unit by using Clean-Trace Surface ATP and Clean- Trace Water ATP tests (3 M, USA) and also obtained samples from bronchoscopes and the environment for smear examination and culture.[6] In our unit automated washing machines are used and high level disinfection is done by ortho-phthalaldehyde (OPA). ATP levels were in acceptable limits and mycobacteria is not detected in those samples. Five of 6 patients remained culture negative and therapy was not given. Among other culture negative patients, 3 of them were trace positive and we have learned that they have had TB treatment previously. In the other two patients, low level PCR positivitiy in BAL samples was supported by clinical and radiological findings therefore they have received TB treatment.

Dorman et al.[5] enrolled 2368 participants for sputum sampling and the study was done at ten reference laboratories in eight countries (South Africa, Uganda, Kenya, India, China, Georgia, Belarus, and Brazil). The sensitivity of Expert Ultra was 63% for the 137 participants with smear-negative and culture-positive sputum. 19 (44%) of 43 participants with a positive Xpert Ultra test but no positive culture had trace and only 2 had M. tuberculosis identified on a follow-up culture . These results are in line with other studies show that Xpert-positive, culture negative results were more common in individuals with a history of tuberculosis.[7,8]

Ultra provides a category which is defined as 'trace' that does not exist with Xpert, corresponds to specimens positive for the PCR targeting the multicopy genes IS6110 and IS1810 and negative for the PCR targeting the single copy gene rpoB.[4] Positive trace result is related with the presence of a very low quantity of *M. tuberculosis* DNA and should be evaluated carefully. Inconsistent culture and PCR results is a big challenge for clinicians. False positive results can be related with amplification of dead bacilli from environmental contamination or previous TB treatment.[5,7] False positivity is related with endoscopic procedure in our study and despite high level disinfection the surface of the bronchoscope remained positive for mycobacterium DNA.

Although the number of clinical samples is not high, every patient counts and we have had difficult time to evaluate our results to guide clinicians. Arend et al., metaphorically speaking defines, Xpert as a knife and Xpert Ultra is a sharper knife and hopes to hear additional studies and algorithms that assess Xpert Ultra's improved sensitivity and solutions to avoid the trap of treating patients for dead bacilli.[9] So far, it is for the best to combine PCR results with radiological and clinical evidence especially when the lack of conventional microscopy and cultivation procedures.

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