Journal of Infection 78 (2019) 491–503

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letters o the Editor

Rapid evolution and gene communication of H3N2 and H1N1 influenza a viruses

Recently, it is reported in Journal of infection that H5N6[1] and H7N9[2] subtype avian influenza virus may have an increased pathogenicity to humans.The H1N1 subtype influenza virus has emerged in China. Not only the H1N1 (95%) subtype but also several H3N2 (5%) subtype influenza viruses have been detected in samples. According to Chinese national influenza data (http://ivdc.chinacdc.cn/), the H1N1 subtype influenza virus was prevalent from the end of 2018 to the beginning of 2019. The H1N1 and H3N2 subtypes of influenza virus are resistant to adamantanes (amantadine and rimantadine), and a small number of H1N1 strains have been found to be less sensitive to NA inhibitors (NAIs; oseltamivir, zanamivir, and peramivir). In this study, we briefly evaluated the evolution patterns of the H1N1 influenza virus and the H3N2 influenza virus.

We collected non-repeat 674 H1N1 and H3N2 subtype influenza virus sequences isolated in China over the course of nearly 5 years from the Global Initiative on Sharing Avian Influenza Data (GISAID) database (www.gisaid.org) and National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/genomes/FLU). The haplotype network map shows that the 2014 H1N1 strain has a node in common with the 2016/17 H3N2 strain (Fig. 1), and H1N1 and H3N2 often co-infect the same patients. When multiple strains of influenza infect the same host, they may undergo recombination and reassortment of the gene fragment, which greatly changes the pathogenicity and epidemiological characteristics of the virus. Currently, the H1N1 subtype influenza virus is widespread in the population, and the number of children with neurological symptoms increased significantly this year.[3] The influenza virus has shown some variation, and whether this variation occurs along H1N1 and H3N2 lines remains to be seen. When multiple viruses co-infection occurs, it becomes possible for the viruses to undergo genetic communication, which may change the direction of viral evolution and so deserves our attention.

We calculated the average gene evolution rate (nucleotide replacement rate) of the H1N1 influenza virus and the H3N2 influenza virus between different years from 2013 to 2019. It can be seen that the genetic evolution rate of the H1N1 influenza virus and the H3N2 influenza virus is $2.91E^{-5}$–$4.03E^{-4}$ and $2.72E^{-5}$–$1.05E^{-4}$ (Table 1) , respectively, in the same year and there are up and down fluctuations that may be related to the subtypes that were prevalent that year. Additionally, we calculated the evolution rate of the H1N1 influenza virus from the end of 2018 to the beginning of 2019 ($1.13E^{-3}$). A large increase in the rate of advancement indicates a rapid change in the virus in the short term, triggering changes in the replication, resistance, and transmission of the virus.

Table 1
The H1N1 and H3N2 subtype influenza virus HA mean rate of nucleotide substitution (substitution/per/year).

(A) The H1N1 subtype influenza virus HA mean rate of nucleotide substitution (substitution/per/year)

2013	$3.61E^{-5}$	95% HPD interval	[$2.4916E^{-5}$, $4.7632E^{-5}$]
2014	$3.69E^{-4}$	95% HPD interval	[$1.6476E^{-4}$, $6.4059E^{-4}$]
2015	$2.91E^{-5}$	95% HPD interval	[$2.0086E^{-5}$, $3.8623E^{-5}$]
2016	$4.03E^{-4}$	95% HPD interval	[$2.1404E^{-4}$, $6.4805E^{-4}$]
2017	$4.24E^{-5}$	95% HPD interval	[$2.6979E^{-5}$, $5.7628E^{-5}$]
2018	$2.40E^{-5}$	95% HPD interval	[$1.7352E^{-5}$, $3.1947E^{-5}$]
2019	$2.58E^{-5}$	95% HPD interval	[$1.9750E^{-5}$, $3.2208E^{-5}$]
2018.11.1–2019.1.31	$1.13E^{-3}$	95% HPD interval	[$5.6521E^{-4}$, $1.6795E^{-3}$]

(B) The H3N2 subtype influenza virus HA mean rate of nucleotide substitution (substitution/per/year)

2013	$1.05E^{-4}$	95% HPD interval	[$5.5226E^{-5}$, $1.6740E^{-4}$]
2014	$4.44E^{-5}$	95% HPD interval	[$2.7470E^{-5}$, $6.0850E^{-5}$]
2015	$3.10E^{-5}$	95% HPD interval	[$2.2854E^{-5}$, $4.0432E^{-5}$]
2016	$2.87E^{-5}$	95% HPD interval	[$1.8451E^{-5}$, $4.0539E^{-5}$]
2017	$2.72E^{-5}$	95% HPD interval	[$1.8271E^{-5}$, $3.7184E^{-5}$]
2018	$4.11E^{-5}$	95% HPD interval	[$3.0029E^{-5}$, $5.2728E^{-5}$]
2019	$2.98E^{-5}$	95% HPD interval	[$1.9811E^{-5}$, $4.0503E^{-5}$]

E: scientific notation.
HPD: highest probability density.

The type A influenza virus emerges periodically every year,Influenza undergoes continuous evolution, and different lineages have appeared.[4] At the same time, under the action of vaccines and drugs, the antigenicity and antigenic site of the virus are transformed and resistant.[5] However, medical science has also improved. It is necessary to determine the frequency of gene exchange between different subtypes and be alert to possible variations in gene communication between different subtypes. Pu et al.[6] and Shi et al.[7] reported that duck-derived virus H7NX and recombination of H7N9 can produce new H7N2 that can cause disease death in waterfowl. This recombination event may have occurred in 2013 or earlier, but the recombinant virus has a distinct evolutionary advantage given the use of a vaccine. Exchange between H1N1 and H3N2 may allow them to give each other different viral characteristics, Hence, ongoing surveillance of H1N1 and H3N2 subtypes of influenza is warranted.

https://doi.org/10.1016/j.jinf.2019.03.009
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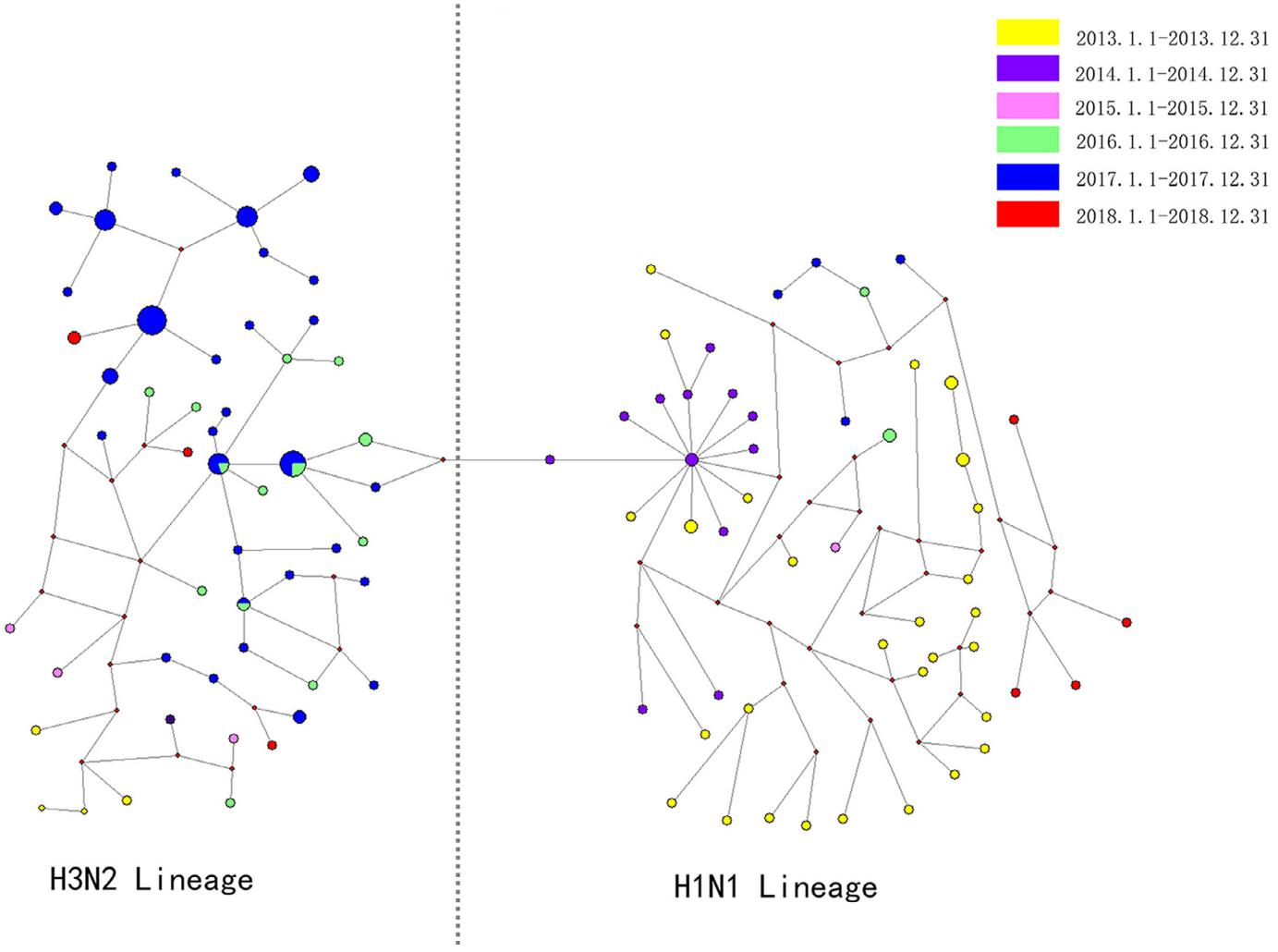

Fig. 1. Phylogenetic network of HA gene for H3N2 and H1N1 AIVs. The median-joint network of HA sequences was constructed with Network 5.0 (http://www.fluxus-engineering.com/sharenet.htm).

Conflict of interest

The authors declare not conflict of interest.

Acknowledgments

This work was supported by China National Natural Science Foundation (31830097), China Agriculture Research System (CARS-41-G16), China National Key Research and Development Program (2016YFD0500800), China National Animal Disease Surveillance and Epidemiological Survey Program (No. 201817), Guangdong Poultry Industry Research System (2017LM1114) and China Scholarship Council Fund Project (201707630003).

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Accepted 19 March 2019
Available online 23 March 2019

https://doi.org/10.1016/j.jinf.2019.03.009

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Human-isolated H7N9 obtained internal genes from duck and human influenza viruses

Dear Editor,

Recent studies in this journal revealed that some H7N9 viruses reassorted with duck AIVs, and then attained the ability to efficiently infect ducks.[1,2] H7N9 AIVs have been endemic in chicken since their emergence in China in February 2013.[3] After its emergence, H7N9 viruses have evolved substantially, and have frequently reassorted, acquiring internal genes from other chicken H9N2 viruses, increasing the genetic diversity of H7N9 viruses.[4] This raises the concern that whether H7N9 can attain internal genes from other AIVs. Thus, we collected all available H7N9 sequences to detect potential novel reassortments of the H7N9 AIVs, and found evidences that three human-isolated H7N9 isolates attained internal genes from duck and human AIVs.

All available sequences of H7N9 AIVs were downloaded from the NCBI (https://www.ncbi.nlm.nih.gov), GISAID (https://www.gisaid.org) and FluDB (https://www.fludb.org) public databases. Then, phylogenetic trees for HA, MP, NP, NS, PA, PB1, PB2 and NA genes were reconstructed, respectively, using RAxML v.8.0.24 with GTRGAMMA model, and 1000 bootstrap tests. Phylogenetic analyses revealed that five genes (NP, NS, PA, PB1, and PB2) of A/Fujian/33845/2017(H7N9), MP gene of A/GD-66/2014/H7N9/2014-01-29, and two genes (PB1 and PB2) of A/Zhejiang/9/2014(H7N9) did not clustered with chicken H7N9 AIVs, respectively (Fig. 1 and Supplementary Fig. 1). Further, Blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search the homology sequences of these three abnormal strains (Table 1). We found that the NP, NS, and PA of A/Fujian/33845/2017 (H7N9) showed very high homology with A/duck/Fujian/13/2013(H1N8) (NP, 98% identity; NS, 99% identity; PA, 98% identity). While PB1

Table 1
Influenza viruses with highest nucleotide identity to each gene of the three new reassortant H7N9 viruses.

Virus Strains	HA	NA	MP	NP	NS	PA	PB1	PB2
EPI_ISL_314056|A/Fujian/33845/2017 (H7N9)	MF280190|A/chicken/Guangdong/Q1/2016 (H7N9) 99%	MF280191|A/chicken/Guangdong/Q1/2016 (H7N9) 99%	KX598701|A/chicken/Guangxi/SIC19/2014(H9N2) 98% MF630160|A/chicken/Guangdong/SD1433/2016(H7N9) 98%	KP658068|A/duck/Fujian/13/2013 (H1N8) 98%	KP658083|A/duck/Fujian/13/2013(H1N8) 99%	KP658053|A/duck/Fujian/13/2013 (H1N8) 98%	KP658098|A/duck/Fujian/13/2013 (H1N8) 94% MH209513|A/duck/Fujian/SD208/2017(H7N9) 99% MH209505|A/duck/Fujian/SD001/2018(H7N9) 99%	KP658008|A/duck/Fujian/13/2013 (H1N8) 94% MH209512|A/duck/Fujian/SD208/2017 (H7N9)99% MH209504|A/duck/Fujian/SD001/2018 (H7N9)99%
EPI_ISL_198688|A/GD-66/2014/H7N9/2014-01-29	MF630061|A/chicken/Guangdong/SD015/2013(H7N9) 99%	MF630063|A/chicken/Guangdong/SD015/2013(H7N9) 99%	KT267001|A/duck/Guangxi/132/2013 (H6N2) 99%	MF630062|A/chicken/Guangdong/SD015/2013 (H7N9) 99%	KP413325|A/chicken/Dongguan/ 3145/2013 (H7N9) 99%	KP865793|A/chicken/Zhejiang/ B2013/2012 (H9N2) 99%	MF630059|A/chicken/Guangdong/SD015/2013 (H7N9) 99%	KX523682|A/Guangdong/7/2013 (H7N9) 99%
		A/Anhui/1-YK_RG25/2013 (H7N9) 99%	AB916662|A/duck/Vietnam/LBM473/2013(H6N6) 99%					KP413392|A/chicken/Dongguan/3418/2013(H7N9) 99%
EPI_ISL_163723|A/Zhejiang/9/2014 (H7N9)[a]	MF630413|A/chicken/Zhejiang/S4135/2013 (H7N9) 98%	–	KP413737|A/chicken/Huzhou/3765/2013 (H7N9) 97%	–	–	–	CY006129|A/NewYork/191/2005 (H3N2)89%	CY002079|A/ New York/396/2005 (H3N2) 88%

[a] *Note:* NA, NP, NS and PA gene sequences of A/Zhejiang/9/2014 (H7N9) are unavailable.

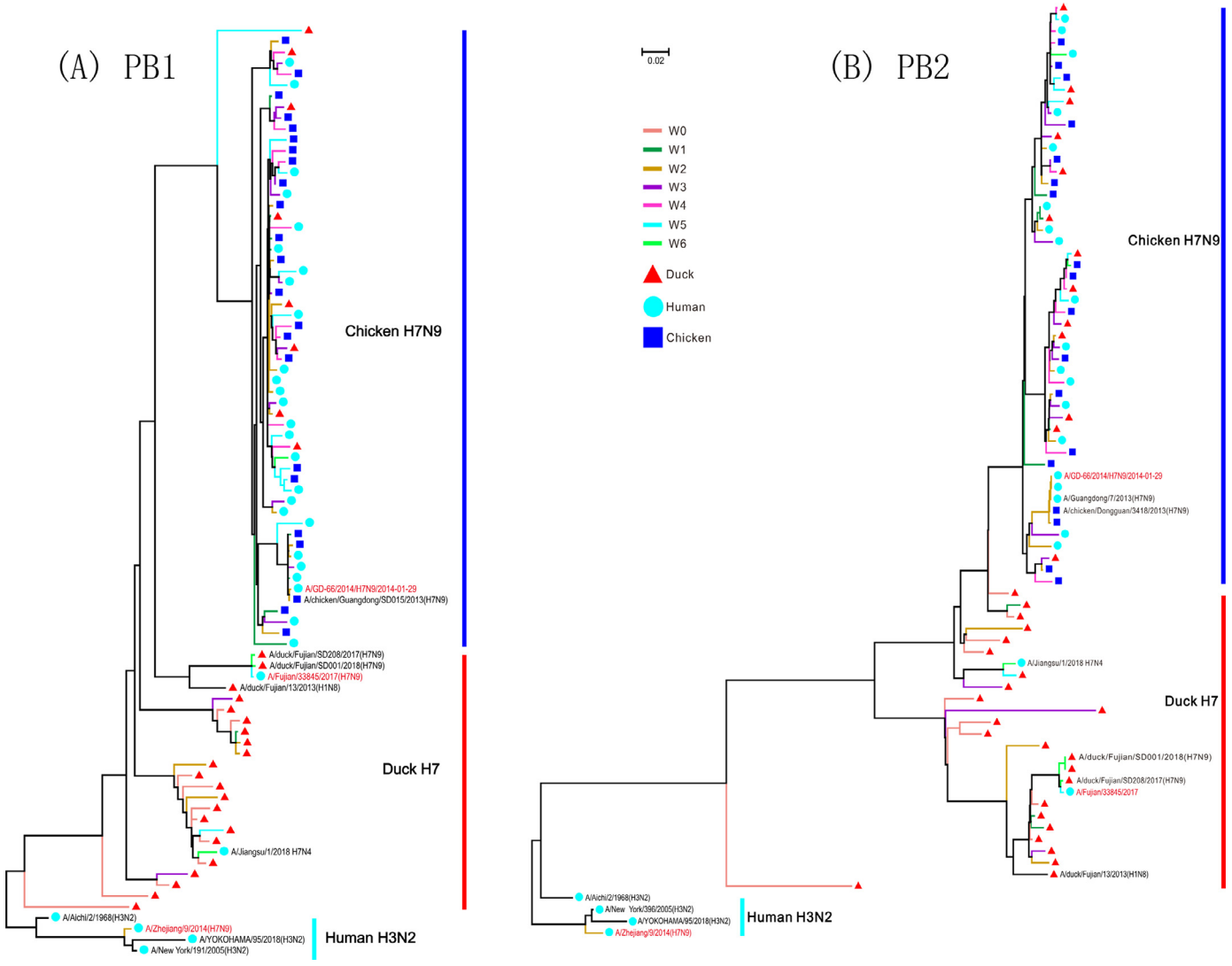

Fig. 1. Phylogenetic trees of the PB2 and PB1 genes of representative viruses. (A) PB1; (B) PB2. Sequences of the three new reassortant isolates are marked in red.

and PB2 genes of this human-isolated H7N9 virus showed high homology with A/duck/Fujian/SD208/2017(H7N9) and A/duck/Fujian/SD001/2018(H7N9) (both of these two duck viruses, PB1, 99% identity; PB2, 99% identity), respectively. The MP gene of A/GD-66/2014/H7N9/2014-01-29 was highly homologous with duck H6N2 and H6N6 AIVs (99% identity). The detections of these two human-isolated H7N9 viruses that derived from the reassortments between 2013 chicken H7N9 and duck AIVs revealed that despite chicken, ducks also play a role in circulation of H7N9 AIVs, and the reassortant viruses even have the ability to cause human infections.

China raises a great number of ducks in open field. Wild waterfowl are natural hosts of AIVs.[5] While domestic ducks act as an interface between the natural gene pool and terrestrial poultry in the influenza virus ecosystem. The 2013 H7N9 viruses cannot replicate efficiently in ducks in the first four waves. However, studies have indicates that the highly pathogenic H7N9 virus has extended its host range by acquiring genes from duck influenza viruses and has now adapted to ducks.[1,2,6] The reassortments between 2013 H7N9 and duck AIVs would further raise the diversity and spread of the H7N9.[7] In addition to our finding of the reassortments between duck AIVs and the human-isolated H7N9 viruses suggest that surveillance and control of duck AIVs is critical for the control of H7N9 viruses, which was almost ignored previously.

It's surprising that the PB1 and PB2 genes of A/Zhejiang/9/2014 (H7N9) showed the most closed relationship with human H3N2 viruses (89% and 88% identity respectively, Table 1). Reassortments between human and avian AIVs can make the reassortant viruses replicate efficiently in mammalian hosts.[8] It's very possible that the reassortment between H7N9 and human H3N2, may make the reassortant virus more adaptive to human, even attain the ability to efficiently transmit between humans, and thus raise great thread to the public health. Historically, several pandemic human influenza viruses were derived from reassortant virus between avian and human influenza viruses. For example, the H1N1/pdm2009 virus, which was a swine reassortant virus that attained the PB1 from human H3N2, was rapidly transmitted between humans and then, globally circulates as a seasonal virus, posing a substantial risk to human.[9] H7N9 AIVs have the genetic makeup associated with human infections,[10] in addition to our finding that the reassortant human-isolated H7N9 virus attained the PB1 and PB2 from human H3N2, possibility like the H1N1/pdm2009 virus, the reassortant virus would become more invasive to humans, and thus pose serious pandemic threat to humans.

H7N9 is a novel reassortant AIV subtype, which has surpassed H5N1 in laboratory-confirmed human infections despite its limited dissemination outside of China. Thus, whether this subtype could acquire the ability to efficiently human-to-human transmission, and become a new influenza pandemic raise great attention. Although a H5/H7 vaccination in chicken has successfully decreased the prevalence of the H7N9 viruses in chicken, our findings that H7N9 viruses attained internal genes from human and duck AIVs raise concerns about the potential ability of the viruses to increase their diversity and spread, and especially the possibility to develop better ability to infect human, and eventually attain efficient human–human infections.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 31822056), Guangdong Natural Science Funds for Distinguished Young Scholar (no. 2014A030306046), and start-up funding from the South China Agricultural University for Yongyi Shen.

Conflict of interest

The authors declare not conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.03.008.

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Accepted 12 March 2019
Available online 23 March 2019

https://doi.org/10.1016/j.jinf.2019.03.008

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Poor transmission of seasonal cold viruses in a British Antarctic Survey base

Dear Editor,

We note with interest these previous studies into household and hospital influenza outbreaks.[1,2] Such community and hospital-based respiratory virus transmission and outbreak investigations often suffer from the potential confounding arising from possible exposures to undiagnosed index cases outside of the outbreak cohort, leading to an overestimate of virus transmissibility, and potentially unnecessary costly and restrictive infection control interventions.

To avoid such confounding, we performed a small pilot study in a closed population of adult research scientists ($n = 43$ out of a possible 48). All participants signed informed consent forms, following ethical approval from Plymouth University Ethics Committee.

These scientists were confined to a British Antarctic Survey base for 1 month (March 2017), during which no personnel entered nor left the base. Therefore any detectable human respiratory viruses could only have been brought into the base by personnel at the beginning of this 'closed period'.

Participants were given anonymous codes to maintain confidentiality. Each agreed to give nasal swabs (collected in virus transport medium, Virocult, Medical Wire and Equipment Ltd, Corsham, Wiltshire, England) upon entry (Day 0, 14/3/17), then at days 4 (18/3/17), 10 (24/3/17) and 17 (31/3/17) post-entry. All viral swabs were stored at −80° C until they could be shipped back to the UK and tested at the Leicester Royal Infirmary. This was performed using a respiratory multiplex PCR assay (16-well, AusDiagnostics UK Ltd., Chesham, UK) that could detect any of: influenza A, B, respiratory syncytial virus (RSV), parainfluenza (PIV) types 1-4, human metapneumo (hMPV)-, entero-/rhino-, corona- (229E, OC43, NL63, HKU1) and adeno- viruses.

No specific instructions about infection control were given to the participants. They were left to act as they would normally behave throughout the period of the study. Any participants who developed any of 9, self-assessed, influenza-like symptoms (fever, cough, stuffy nose and/or sinuses, headache, sore throat, myalgia, fatigue, shortness of breath, nausea or vomiting) would complete a tick-box questionnaire (on a scale of 1-'very mild' to 5-'very severe') to describe the relative severity of their symptoms.

This same questionnaire also requested the contact intensity (i.e. number, nature and frequency) of their daily contacts with other participants as a self-assessed, linear graded score (from 1-

'sharing just one meal together' to 5-'spending the majority of the day and evening with the other person'), depending on the frequency of contact whilst working, eating meals and socialising together. The daily location of all personnel in any of the four station zones at 0830, 1100, 1400, 1630 and 2000 h was also recorded routinely for safety and security, using a 'tagboard' system.

Out of the 43 participants who consented, 3 later declined to have any viral swabs taken, and of the resulting 160 (i.e. 40×4 swabbing time-points) possible swabs, 153 were successfully collected and stored for testing. Testing revealed 2 positive results: on Day 4, Participant 21 - coronavirus NL63 positive; on Day 7, Participant 74 - coronavirus OC43 positive.

Five symptomatic participants completed questionnaires: on Day 0, by Participants 40 and 47; on Day 2, by Participant 21 (linking to the Day 4 coronavirus NL63 positive result); on Day 7, by Participant 74 (linking to the Day 10 coronavirus OC43 POS); on Day 17, by Participant 107. The self-reported symptoms consisted of mainly fatigue and sore throat, with/without some degree of 'stuffy nose and/or sinuses'.

The incubation period of human coronaviruses is around 2-5 days,[3] which can be used to link symptomatic cases together, epidemiologically,[4] with viral shedding being reported for up to 6 days post-symptom onset.[5] So the symptoms and positive NL63 and OC43 results for Participants 21 and 74, respectively, could have been acquired from Participants 40 and 47, who may have been the original index cases (sources) for these viruses. Although no respiratory virus was detected in their samples, Participants 40, 47 and 107 all reported similar symptoms to those of 21 and 74 during the study period, which were typical common cold symptoms.

Note that the participants' self-reported contact intensities were not entirely robust, e.g. both Participants 40 and 47 list Participant 21 as a contact, so could both have been index cases for him/her. However, 21 did not list either 40 or 47 as a contact (such contacts should be reciprocal). This may have just been a simple oversight, but it makes the contact link less reliable. Similarly, Participant 74 could have served as the index case for Participant 107, but neither lists the other as a contact. Regardless of the contact intensities reported in the questionnaires, there were no secondary cases of either NL63 or OC43 coronaviruses detected in any of the other study participants' weekly swabs. One possible explanation for this may have been an insufficient sensitivity of the assay to detect low levels of these respiratory viruses.

The limit of detection (LOD) for the AusDiagnostics assay varies significantly with each virus (as given in the kit insert, all in copies/ml): influenza A (1900-2375), B (525), RSV (50-2125), PIV types 1-4 (50-2500), hMPV (100-625), entero-/rhino- (75-1025), corona- (229E, OC43, NL63, HKU1) (1350-4175) and adeno- (1075) viruses. However, in the acute infection stage respiratory viruses are generally present in relatively high copy numbers, with median values of mostly 4-8 $\log_{10}$ (i.e. 10,000-100,000,000 copies/ml) for adeno-, corona-, hMPV, influenza, PIV and RSV, as reported in one comprehensive paediatric study.[6] Although children generally shed higher viral loads than adults, it is likely that the coronavirus loads in acutely infected adults would still be mostly detectable on this assay, which is approved (i.e. CE-marked) for routine diagnostic testing. Yet, it is still possible for viruses that are infecting individuals at the lowest loads within these ranges, to fail to be detected by this assay.

Given the results that are currently available from this study, one of the key questions is: from where did the NL63 and OC43 coronaviruses arise? In addition, the lack of any secondary NL63 or OC43 coronaviruses cases (symptomatic or asymptomatic) arising from the known positive sources (Participants 21 and 74), suggests that the transmissibility of these common cold viruses may be limited. This seems unexpected, given the potential stress on the body immune system whilst living and working in such an extreme environment. However, such relatively poor transmission of respiratory viruses has been previously described in Antarctic base personnel for rhinovirus and adenovirus,[7,8] for reasons that are still unclear.

Another potential confounding factor is the unknown status of the 5 individuals who were also present at the base (mostly base personnel) but who declined to participate in the study. It is possible that one or more of these non-participants could have been the original sources (i.e. index cases) of the NL63 and OC43 coronaviruses at the start of the study.

Whilst there are some limitations to this study, there are plans to repeat this on a larger scale, over a longer 'closed' period, with the use of real-time, point-of-care testing (POCT) to detect such respiratory viruses. Although previous respiratory virus outbreaks have been described in remote research bases,[7,8] these were unable to utilise the greater sensitivity and spectrum of respiratory viral targets provided by modern, molecular, diagnostic tools.[9,10] Thus, some positive cases in these earlier studies may have been missed, leading to an underestimate of the transmissibility of these respiratory viruses in these populations.

Respiratory infections in research personnel can impact significantly on their productivity, an important consideration when their time at such remote research bases is limited. This and future studies will enable medical teams to enhance the healthcare of research base personnel to optimise their precious research time spent there.

Conflicts of interest

None of the authors have any conflicts of interests to declare.

We thank the following for their support of this study: UK Clinical Virology Network (CVN), for general funding support; Medical Wire & Equipment Ltd., for donating some of the sampling swabs; Ausdiagnostics UK Ltd., for donating the respiratory multiplex PCR tests. None of these companies were involved in the writing of this article.

Acknowledgments

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Accepted 12 March 2019
Available online 23 March 2019

https://doi.org/10.1016/j.jinf.2019.03.007

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First experience of ribavirin postexposure prophylaxis for Nipah virus, tried during the 2018 outbreak in Kerala, India

Dear Editor,

We read with interest, the article by Poller et al.[1], in this journal, entitled “A unified personal protective equipment….”. We understand the importance of proper personal protective equipment(PPE) as an integral component of healthcare workers(HCW) protection in outbreak situations of infections with possible high consequence. But at times, such an outbreak occurs in an unsuspected region, when initial cases present in early course of illness before the development of ominous clinical features. Medical staff, particularly in busy rural set ups of resource-poor developing countries may discover that they have been exposed to a high consequence infectious disease after the event of exposure, particularly if these centres are unaware, reluctant or unequipped regarding routine use of PPE. A similar situation occurred in a rural healthcare setting of Kerala, India, during the May-2018 outbreak of Nipah virus (NiV), killing 21 out of 23 reported cases.[2]

A 26-year-old male (*index case* of the outbreak report[2]) from Kerala’s Perambra town died undiagnosed with fever, encephalitis and respiratory distress in Government Medical College Kozhikode(GMCK), after being transferred from Taluk Hospital, Perambra(THP). Another 47-year-old male patient (*Case-10*)[2] was admitted in THP for an acute febrile illness and recovered while the unsuspected *index case* was being treated there in the adjacent bed. Two weeks later, *Case-10* presented to Taluk Hospital, Balussery(THB) (the setting of our intervention), with complaints of fever, headache and vomiting of 4 days duration. He was treated there as inpatient for about 24 h after which he was referred to GMCK, owing to clinical deterioration. The next day he developed altered sensorium, respiratory distress and expired.

Till then, there was no suspicion about the NiV outbreak situation, as Kerala is at least 2500 km away from the last known outbreak in the Indian subcontinent in 2012[3,4]. In the meanwhile, the brother, father and aunt of the *index case*, and a nurse, who cared for him at THP, developed similar clinical features of acute encephalitis with respiratory distress and got admitted. All of their samples, along with that of *Case-10*, were tested positive for NiV from the reference laboratory. The state public health authorities swiftly declared the outbreak and ensured containment and protective measures. However, by this time, 17 out of 18 confirmed NiV cases were already infected and fell ill, being epidemiologically related as contacts of the *index case* in family, during transit to healthcare facilities or in hospital[2].

Here, we report our experience with eight HCW including two doctors (authors APS and MB of this correspondence) and six nurses working in THB, who had unsuspected, inadvertent, yet significant exposure to *Case-10* when he was admitted there, without any PPE. Both the doctors had closely clinically examined *Case-10* and the six nursing-staff had repeated bare-hand, unmasked contacts with him (Table 1). All of them were extremely panicked once the outbreak notification was out and beseeched APS and MB for an immediate solution. APS and MB contacted the other authors for advice regarding any possible post-exposure prophylaxis(PEP).

Considering the possibility of human-to-human (airborne / contact) transmission of NiV,[3,4] in-vitro[5] and in-vivo[6] effects of ribavirin on NiV, evidence of safety and efficacy of short-course high-dose ribavirin PEP(rPEP) used for Lassa fever[7] and unavailability and inexperience of any other alternatives (favipiravir or monoclonal antibody m102.4), were discussed by VKMN, SB, MS, NW and AB, and a consensus opinion of rPEP as the only available and reasonably safe option was placed before APS and MB. The importance of psychological factors[8] in the HCWs were also considered seriously. The suggested dose was 1000 mg thrice daily for 14 days(cumulative 42,000 mg) in congruence to the Lassa fever recommendation.[7] All the contacts started rPEP within 72 h of exposure. The mean cumulative dose of rPEP taken by the contacts was 28,750 mg(17,600–40,000 mg) and the mean duration was 12.5 days(11–14 days, Table 1). Their clinical and laboratory parameters were monitored for the next 6 months. Mean age of the 8 HCWs was 35.4 years(30–43 years). Two were males and rest females; none were pregnant (Table 1). Most of them experienced minor side effects like fatigue, headache, nausea, dry mouth and palpitations. There was a mean drop of 2.82 g/dL of haemoglobin, predominantly between days 17 and 21 after starting rPEP, which started rising in all within a week of stopping rPEP. Bilirubin levels rose by a mean of 1.65 (range: 0.0 – 3.6) mg/dL in 7 of the 8 HCW (Fig. 1). None of them ultimately contracted NiV disease.

Interestingly, one 25-year-old male patient (*Case-23* of the outbreak report[2]), was admitted at an adjacent bed in THB with dysentery, while *Case-10* was admitted. He was present within a distance of 1 metre for more than 5 h. There was apparently no direct contact between them, except that same HCW served both of them sharing non-critical medical devices. After recovery, *Case-23* was discharged from THB, to return after a week to

Table 1
Profile of the exposed healthcare workers.

	Age in yrs/Sex	Degree of exposure	Use of personal protective equipment	Duration of exposure	Cumulative dose of Ribavirin taken (mg)	Reason for deviation from suggested dose	Symptoms after starting on Ribavirin
Doctor 1	39/M	Thorough clinical examination of the patient	No	45 min	40,000	Raise in Bilirubin	No symptoms
Doctor 2	31/M	Thorough clinical examination of the patient	No	15 min	27,400	Raise in Bilirubin, fever	Fever, fatigue, dizziness, anorexia, nausea, palpitation and exertional dyspnoea
Nurse 1	34/F	Assisted the doctor during examination. Inserted intravenous (IV) cannula for the patient. General patient care and bedding change.	No	40 min	24,800	Raise in Bilirubin, drop in haemoglobin	Nausea, fatigue.
Nurse 2	30/F	Administered IV medications, obtained a few samples, general patient care and bedding change	No	40 min	38,200	Afraid of potential adverse effects	Fatigue, headache
Nurse 3	42/F	Administered IV medications, obtained a few samples, general patient care and bedding change	No	25 min	25,800	Afraid of potential adverse effects	Fatigue, headache, anorexia
Nurse 4	34/F	Changed IV infusion and administered IV antibiotics and cared for positioning and checking blood glucose and temperature	No	20 min	35,600	Raise in Bilirubin	Fatigue, headache, anorexia, nausea, malaise, dysgeusia
Nurse 5	43/F	Changed IV infusion and administered IV medications	No	15 min	20,600	Raise in Bilirubin	No symptoms
Nurse 6	30/F	Checked blood glucose, temperature, and vitals, administered IV medications	No	10 min	17,600	Raise in Bilirubin	Fatigue, dry mouth

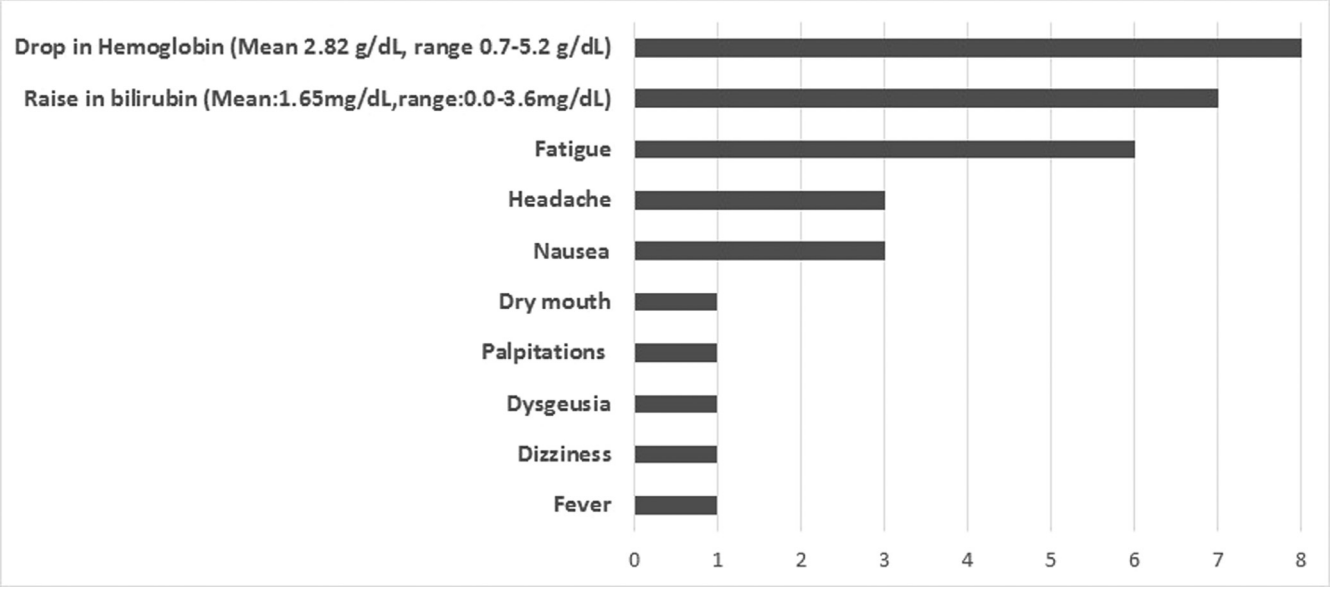

Fig. 1. Adverse reactions of short-course high-dose ribavirin.

GMCK with high grade fever, developing encephalopathy and respiratory distress, diagnosed to have NiV infection and succumbed to it.

In the current outbreak, 3 family members, one staff nurse, one trainee nurse, one radiology assistant who took care of the *index case* and 13 hospital contacts contracted the infection, proving human-to-human transmission. Respiratory aerosols and fomites cause human-to-human spread.[3] The mortality rate, in all recent NiV outbreaks in the Indian subcontinent is 75–100% with the Bangladeshi strain[3] (NiV-B) and its close relative in the recent Kerala outbreak,[2] has been consistently almost double compared to Ebola. Further that *Case-23* getting infected from *Case-10* in the same premises of THB, makes our case for rPEP stronger. The 2 survivors out of 23 infected patients in this outbreak have also received ribavirin.[9]

Given the recent findings of widespread presence of NiV among *Pteropus* bats in India,[10] another outbreak might be just a matter of time. Our field notes from emergency, voluntary, off-label rPEP among HCW provides evidence, albeit low-quality, of its safety and probable efficacy, strongly suggesting a pre-planned trial for PEP to be started immediately once such an explosive outbreak of NiV is notified.

Conflicts of interest

None.

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Accepted 3 March 2019
Available online 23 March 2019

https://doi.org/10.1016/j.jinf.2019.03.005

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Managing monkey bites in returning travellers

Dear Editor,

We note the previous report describing a decreasing incidence of eosinophilia in returning travellers by Barrett and colleagues.[1]

In contrast, another type of hazard reported by returning travellers – monkey bites – appears to be increasing. This makes it necessary for our frontline medical staff to be aware of the potential risks from rabies and simian herpes B virus (SHBV or Cercopithecine herpesvirus 1 – CeHV-1) associated with this type of exposure.[2]

Although infections are rare as a consequence of bites,[3,4] both viruses can result in very high (80-100%) mortality if the appropriate post-exposure prophylaxis is not initiated promptly. In view of these serious consequences, post-exposure protocols have been developed to reduce likelihood of infection.[2,5–7] While this is agreed for rabies,[5,8] post-exposure prophylaxis is not uniformly recommended for SHBV, as cases have only been reported with captive monkeys,[3,9] despite numerous monkey bite exposures in regions where animals are thought to be infected. Assessing risks versus benefits obviously needs to be done judiciously in each case, and national public health specialists can be consulted to support decision making.[8,9]

The main risk is primarily from monkey bites from macaque monkeys (genus *Macaca*), which are now encountered relatively frequently in various tourist areas in Southeast Asia (e.g. Philippines, Indonesia, Malaysia, Cambodia, Vietnam, Thailand).

After a monkey bite, the patient should perform immediate wound cleansing: irrigation with soap and water, or other skin-cleansing detergent, or sterile water alone, for at least 15 min. Later, when the patient presents to the emergency department (ED), all medical staff need to be aware of both the rabies and SHBV post-exposure protocols (PEPs) associated with such bites. Whilst most ED teams will likely know of the rabies PEP protocol,[5] fewer will be aware of the guidelines for SHBV PEP.[6]

Along with the wound cleansing and post-exposure rabies immunoglobulin (RIG) and vaccination, any risk of SHBV requires that high dose acyclovir (preferably valaciclovir 1 g TDS PO; or acyclovir 800 mg 5 times daily PO, for adults) PEP for at least 14 days should be considered. Immediate PCR and later serological testing for signs of SHBV infection are possible. However, recommendations for such testing are somewhat variable, with some advising testing in symptomatic cases only, whilst others will test all potentially exposed cases, regardless of symptoms.[6,7]

Symptoms of possible SHBV disease include vesicular lesions, pain and itching near the bite site, local lymphadenopathy, flu-like illness (fever, headache, myalgia, fatigue), and any focal or progressive neurological symptoms, including dyspnoea.

Outcomes are generally fatal (80% mortality without any treatment), once there is central nervous system involvement.[2,7] However, with antiviral prophylaxis and treatment, such fatal outcomes are rarer. Bacterial infections (e.g. *Staphylococcus* and *Streptococcus* spp.) can also arise from the bite itself, especially in children, for which systemic antibiotics can be given,[10] and tetanus vaccination.

To highlight this issue, we present three cases of returning travellers with monkey bites.

Case 1: A 24-year old male was admitted with headache, lethargy and myalgia following a trip to Indonesia (Monkey Forest, Ubud, Bali), where he sustained a penetrating bite to his right shoulder from a macaque monkey. There were no immediate post-bite complications. However, 16 days later, he developed paresthesia and neuropathic pain in his right thigh. After seeing a local physician, oral aciclovir 800 mg 5 times daily for 14 days was prescribed, as prophylaxis for possible SHBV. One day later he

developed a vesicular rash on his right thigh, which subsequently resolved on the antiviral therapy.

On return to the UK, given this history, he was admitted and started rabies post-exposure immunisation, without rabies immunoglobulin (RIG). He was then extensively investigated for possible SHBV infection. He had five days of intravenous aciclovir and a further nine days of oral valaciclovir, whilst awaiting investigation results.

Diagnostic testing performed at the Department of Viroscience, Erasmus Medical Centre (M/C), Rotterdam, The Netherlands on cerebrospinal fluid (CSF), blood, lesion swab and saliva by SHBV PCR showed no evidence of infection at that time.

At outpatient review, two months later, there had been no further history of any rash or neurological symptoms, though the patient did mention several recurring episodes of genital herpes, for which he was given a 10-day course of oral valaciclovir 500 mg BD PO. By this time, the risk of latent SHBV was considered negligible and he was discharged from clinic.

Case 2: A 28-year old male was seen in clinic who gave a history of receiving an unprovoked, penetrating bite on his right upper arm from a vervet monkey (*Chlorocebus pygerythrus*, previously classified as *Cercopithecus aethiops*), one week earlier whilst on holiday in Barbados. After immediate wound care, he was seen in a local clinic and received tetanus vaccine and oral antibiotics. No aciclovir SHBV PEP was commenced at this time. The bite wounds healed without complication.

After returning to the UK a week later, an outpatient review revealed that he was still asymptomatic for any clinical features of SHBV, rabies or other travel-associated illnesses. However, as a precaution, valaciclovir 1 g TDS PO, for 21 days was prescribed as SHBV prophylaxis, due to the possibility of an incubating SHBV infection.[3]

Blood and saliva samples were sent to Viroscience for PCR testing to check for any residual SHBV, dengue, Chikungunya or Zika virus infections. All tests were negative. The patient continued to remain asymptomatic, so was eventually discharged from outpatient follow-up.

Case 3: A 31-year old female was seen in clinic upon return from Southeast Asia, with a history of receiving a penetrating bite to her right upper arm from a macaque monkey, whilst visiting Monkey Island, Vietnam, 18 days previously. She received her first dose of rabies vaccination (without RIG) at a local clinic within four hours of the bite. A week later, whilst still in Vietnam, she received a second dose of rabies vaccine from a different clinic, which also started her on acyclovir post-exposure prophylaxis for SHBV. The bite wounds healed without sequelae. At her 18-day clinic review once back in the UK, she was still asymptomatic. Baseline saliva and blood samples were taken and stored but not tested for SHBV. She continued both the SHBV and rabies PEP whilst continuing her travels a week later, and remained asymptomatic six weeks post-exposure.

This small case series demonstrates a diversity of presentations and follow-up management for these patients, notably: Case 1 likely presented with genital herpes; Case 2 sustained a bite from a vervet, not a macque, monkey; Case 3 did not have any SHBV testing. To our knowledge, all of these cases remain well.

As there are no consensus guidelines available for managing such monkey bites, we suggest a precautionary approach and that to be safe, assume that both rabies and SHBV are potential risks, regardless of monkey species. Therefore, in the event of a monkey bite, where, after discussion with the patient (based on their individual clinical assessment), a decision is made to give prophylaxis:

(i) Immediately cleanse the wound for 15 mins with clean water +/− soap or detergent. Consider appropriate antibiotic therapy to prevent skin infection (e.g. co-amoxiclav or amoxicillin), and tetanus vaccination.

(ii) Seek competent clinical help and obtain the first dose (Day 0) of rabies vaccine +/− RIG, depending on the risk assessment (in the UK, Public Health England tel: 0208 327 6204, 9–5 pm Mon–Fri).[8] Complete post-exposure rabies vaccination with further doses on Days 3, 7 and 21, post-exposure.[5]

(iii) Start acyclovir (1 g valacyclovir TDS PO or 800 mg acyclovir 5 times daily PO – depending on local availability, for adults. Adjust the dose as appropriate for children) as soon as possible after the bite, for at least 14 days, as post-exposure prophylaxis against SHBV.

(iv) If symptoms compatible with SHBV develop within the next 2–4 weeks (e.g. vesicular lesions, pain, itching around the bite, local lymphadenopathy, flu-like illness, focal or progressive neurological symptoms), continue the acyclovir and seek further expert advice.

(v) Baseline samples (serum, saliva, wound swabs) can be taken and stored for comparison. If acute illness develops, repeat samples (including cerebrospinal fluid – CSF) should be taken for diagnostic testing (by PCR) to check for SHBV DNA, and serology for SHBV antibodies – if such testing is available.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

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Accepted 1 March 2019
Available online 23 March 2019

https://doi.org/10.1016/j.jinf.2019.03.002

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The dengue outbreak of 2014 transformed the epidemic characteristics of dengue in Guangdong Province, China

Recent article in this Journal has reported dengue patients were associated with a higher risk of autoimmune diseases than non-dengue patients[1]. Dengue has been a serious public health problem in the world, the number of dengue epidemics has been on the rise worldwide. Before 1970, only nine countries had experienced severe dengue epidemics; however, currently more than 100 countries have been severely affected by dengue[2]. After the first dengue-fever epidemic in China, which occurred in May 1978 in Foshan, Guangdong Province, there have been regional outbreaks of dengue every year and the number of cases has increased. Guangdong Province is the area most seriously affected by dengue in China. The number of cases in Guangdong accounts for more than 90% of the total number of cases in China [3,4]. However, the epidemic characteristics of dengue fever in Guangdong Province have not been reported since 2010[5].

A dengue epidemic is closely related to various factors, such as environmental conditions, imported cases, and migration; thus, its epidemic characteristics and patterns change rapidly. Especially after the outbreak of dengue in Guangdong Province in 2014, the epidemic patterns have changed greatly. Therefore, exploring the changing patterns of dengue outbreaks and epidemics in Guangdong is of great significance to the prevention and control of dengue. This study aimed to investigate the changing patterns of the epidemic characteristics of dengue in Guangdong Province and to propose prevention and control measures.

We collected dengue cases from 2008 to 2017 from the web-based disease reporting information system of the Chinese National Center for Disease Control and Prevention. A total of 52,792 cases were reported for this period. Population data were obtained from the Statistics Bureau of Guangdong Province (http://www.gdstats.gov.cn/). A Chi-square test was used to determine spatial differences in cities. This study was approved by Center for Disease Control and Prevention of PLA Review Board.

In terms of temporal distribution (Fig. 1), dengue cases showed an increasing trend from 2008 to 2014 in Guangdong Province, and a decreasing trend from 2014 to 2017 (93 cases in 2008, 45,170

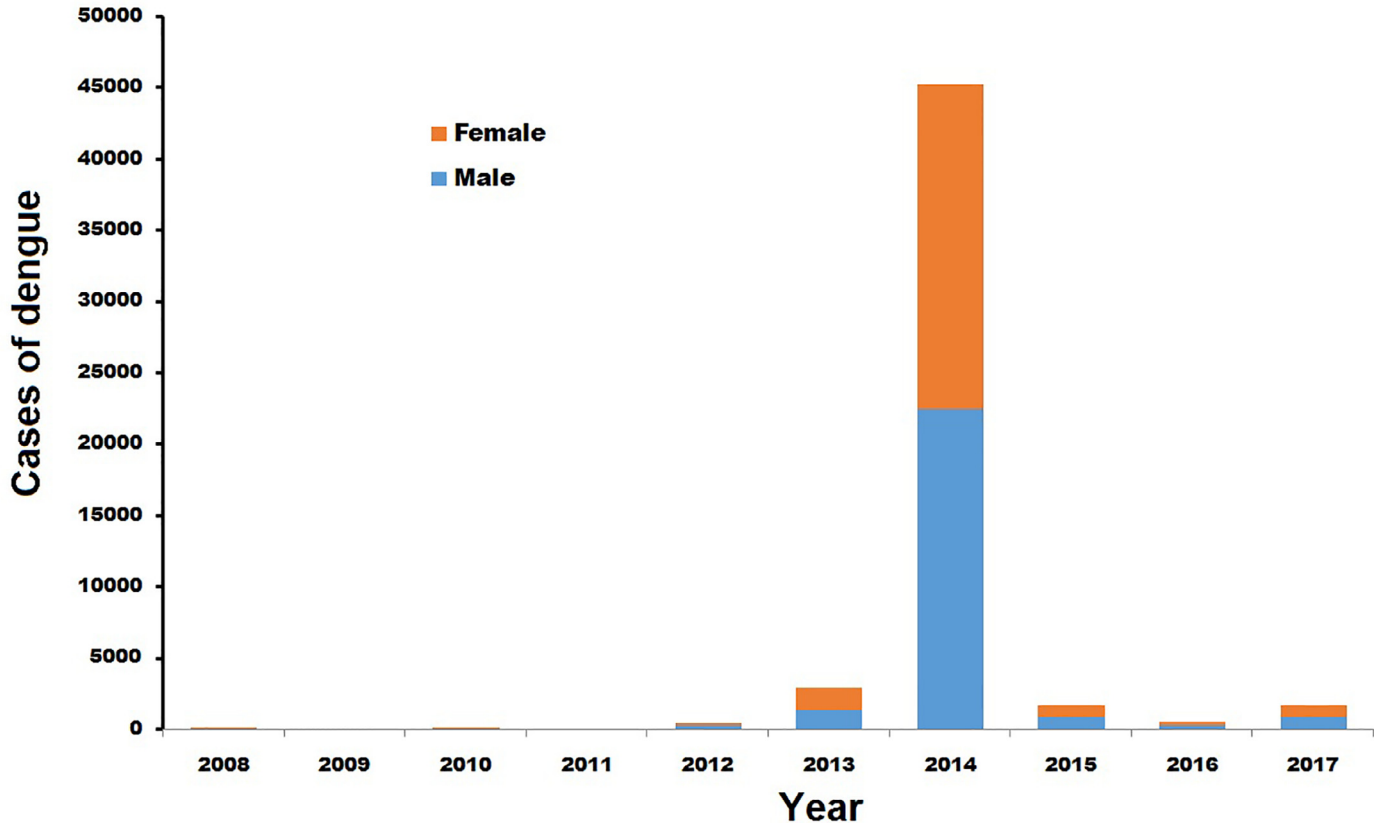

Fig. 1. Temporal distribution of dengue in Guangdong Province (2008–2017).

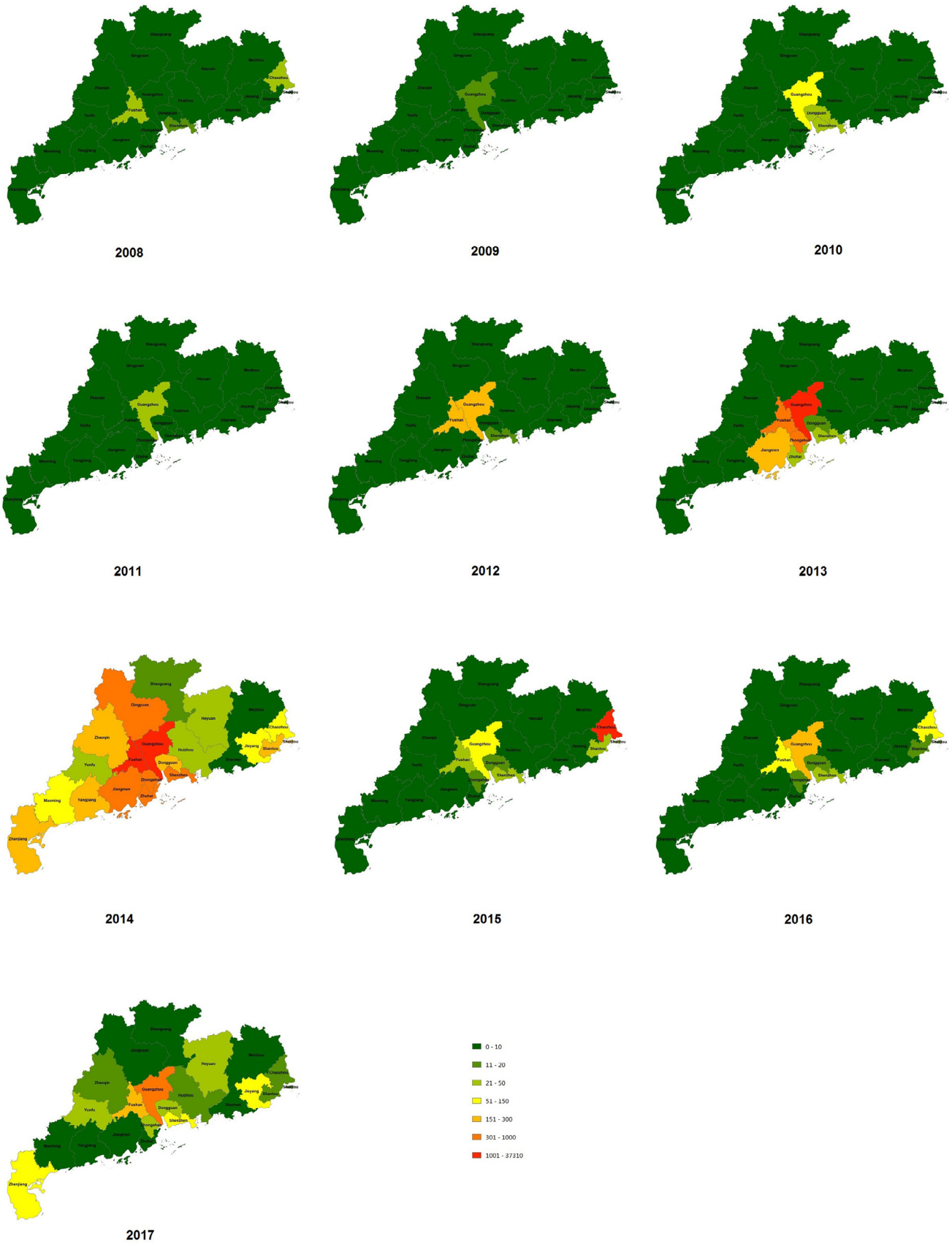

Fig. 2. Spatial distribution of dengue in Guangdong Province (2008–2017).

cases in 2014, and 1662 cases in 2017). The dengue outbreak in 2014, with 45,170 cases and 6 deaths, was the largest dengue outbreak in China in the past 20 years. Before the outbreak in 2014, an average of 617 people was infected with dengue annually. Following the outbreak, an average of 1305 people were infected with dengue annually—nearly twice the number of cases per year compared to that before 2014. Dengue fever had typical characteristics in population distribution. Adults aged 20–65 years accounted for 76.76% of patients, and children aged 0–10 years only accounted for 4.16% of patients.

In terms of distribution across occupations, housekeepers and the unemployed (23.1%), retired individuals (12.82%), those involved in commercial services (11.52%), industrial worker (9.56%), and students (7.49%) formed the majority of patients with dengue fever. These five groups accounted for 64.48% of the total number of cases. The number of male patients was comparable to that of the number of female patients (male: female=1:1.01), and the mortality rate of dengue was low at only 0.11‰. Monitoring data of the past 10 years showed that although the number of cases increased greatly after the outbreak in 2014, the population distribution did not change significantly. The key population for the prevention and control of dengue fever were adults over the age of 20 years.

Although cases have been reported in various regions, there are significant differences ($P<0.001$) in the distribution of dengue among the different cities in the Guangdong Province (Fig. 2). Incident cases were mainly concentrated in Guangzhou City and Foshan City. Guangzhou City had a total of 40,170 cases during 2008–2017, which accounted for 76.09% of cases among the different cities. Foshan City had a total of 4660 cases, which accounted for 8.83% of cases. However, according to annual data trends, 2014 was the turning point in spatial distribution patterns for dengue fever. From 2008–2013, the incidence of dengue was concentrated in Foshan City, Guangzhou City, Jiangmen City, and Zhongshan City (93.14% of cases). However, by 2014, except for Meizhou City, there were case reports from all 20 cities in the province. Among them, there were 13 cities with more than 200 cases each. After 2014, dengue fever was prevalent throughout the province. Even in Zhanjiang City, Zhaoqing City and Jieyang City, which are distant from Guangzhou City, and the number of cases in remote areas increased significantly.

This paper is the first to analyze the characteristics of the change in dengue epidemics in Guangdong Province in the past 10 years. In general, the incidence of dengue fever has typical spatial differences. The cases were mainly concentrated in urban areas with large populations and developed economies, such as Guangzhou City and Foshan City. Theinflux of many migrants, especially those from Southeast Asia, could have caused the higher incidence of dengue fever in these areas. Studies have shown that dengue epidemics in China were initiated by imported cases and then became prevalent in the local areas[6]. However, attention should be given to the large number of dengue epidemics in remote areas, which appeared after 2014. A possible reason for this could be global climate warming, which has caused the natural environments in some remote areas to become suitable for mosquito breeding. Moreover, rapid development in tourism and trade has also led to an increase in local and imported cases. In summary, after 2014, the epidemic characteristics of dengue fever in Guangdong Province have undergone major changes. The key areas for prevention and control are no longer confined to traditional epidemic areas, such as Guangzhou City and Foshan City. Instead, dengue prevention and control should be conducted throughout the province. In addition, according to the national dengue surveillance in 2018, dengue fever has shown a trend of “moving up north” in the country[7]. Therefore, the changing characteristics of this epidemic warrant high attention of relevant departments.

Potential conflicts of interest

The authors declare that they have no competing interests.

Funding

This work was supported by National Key R&D Program of China (2016YFC1200700), Beijing Nova Program (Z171100001117102) and Military Medical Innovation Project (16CXZ046) and PLA Youth Training Project for Medical Science (16QNP127). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Accepted 1 March 2019
Available online 23 March 2019

https://doi.org/10.1016/j.jinf.2019.03.001

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