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GISAID: Global initiative on sharing all influenza data – from vision to reality

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Ten years ago, a correspondence [1,2], signed by more than 70 championed '*A global initiative on sharing avian flu data*' (GISAID) [3], leading to the GISAID Initiative in 2008. What started out as an expression of intent to foster international sharing of all influenza virus data and to publish results collaboratively has emerged as an indispensable mechanism for sharing influenza genetic sequence and metadata that embraces the interests and concerns of the wider influenza community, public health and animal health scientists, along with governments around the world. Today GISAID is recognised as an effective and trusted mechanism for rapid sharing of both published and 'unpublished' influenza data [4]. Its concept for incentivising data sharing established an alternative to data sharing via conventional public-domain archives.

In 2006, the reluctance of data sharing, in particular of avian H5N1 influenza viruses, created an emergency bringing into focus certain limitations and inequities, such that the World Health Organization (WHO)'s Global Influenza Surveillance Network (now the Global Influenza Surveillance and Response System (GISRS) [5]) was criticised on several fronts, including limited global access to H5N1 sequence data that were stored in a database hosted by the Los Alamos National Laboratories in the United States (US) [6,7]. This data repository, set up with financial support from the US Centers for Disease Control and Prevention (CDC) as a first attempt to share 'sensitive' data from affected countries, but was accessible only to those who were also providing H5N1 sequence data. This limited-access approach restricted wider sharing of data prior to publication, which was vital for broader understanding of the progress of the emergent public and animal health threat. The need for greater transparency in data sharing and for acknowledgement of those contributing samples from H5N1-infected patients and animals and related genetic sequence data was not satisfied by sharing data after formal publication via public-domain databases. Scientists charged with the day to day responsibilities of running WHO Collaborating Centres (CCs) for Influenza, National Influenza Centres and the World Organisation for Animal Health (OIE)/ Food and Agriculture Organization of the United Nations (FAO) [8] reference laboratories, were therefore eager to play a key role and provide scientific oversight in the creation and development of GISAID's data sharing platform that soon became essential for our work.

A unique collaboration ensued, involving, in addition to members of WHO's GISRS and OIE/FAO reference laboratories, the wider influenza research community along with officials in governmental institutions and non-governmental organisations. Facilitated by a well-connected broadcast executive with background in licensing of intellectual property, an agreement was drawn up on the sharing of genetic data to meet emergency situations, without infringing intellectual property rights - the GISAID Database Access Agreement (DAA). The DAA governs each individual's access to and their use of data in GISAID's EpiFlu database [9]. It was this alliance between scientists and non-scientists, with a diversity of knowledge and experience, involved in drawing up an acceptable simple, yet enforceable, agreement which gained the trust and respect of the scientific community and public health and animal health authorities.

The essential features of the DAA encourage sharing of data by securing the provider's ownership of the data, requiring acknowledgement of those providing the samples and producing the data, while placing no restriction on the use of the data by registered users adhering to the DAA. It essentially defines a code of conduct between providers and users of data, cementing mutual respect for their respective complementary contributions, and upholding the collaborative ethos of WHO's GISRS, initially established 65 years ago this year [5].

Launched in 2008, the EpiFlu database was of key importance in the response to the 2009 influenza A(H1N1) pandemic, allowing countries to readily follow the evolution of the new virus as it spread globally [10]. Acceptance of the GISAID sharing mechanism by providers and users of data, and the confidence of the influenza community, were further illustrated in 2013 by the unprecedented immediate release of the genetic sequences of Influenza A(H7N9) viruses from the first human cases, by Chinese scientists at the WHO Collaborating Centre for Influenza in Beijing [11,12]. Such events reaffirmed GISAID's applicability to timely sharing of crucial influenza data. The subsequent use of the sequence data to generate, develop and test candidate vaccine viruses by synthetic biology within a few weeks also demonstrated how GISAID successfully bridged this important 'technological' gap [13,14]. The paper by Bao et al. from Jiangsu province of China published in this issue once again confirms the importance of the timely sharing of data on the evolution of the A(H7N9) viruses for global risk assessment. The authors analysed the recently isolated H7N9 viruses form the fifth wave in Jiangsu province, and the results showed no significant viral mutations in key functional loci even though the H7N9 viruses are under continuous dynamic reassortment and there is genetic heterogeneity. These findings should help to reduce concerns raised, even though the number of human infection with H7N9 virus increased sharply during the fifth wave in China.

GISAID provides the data-sharing platform particularly used by GISRS, through which sequence data considered by the WHO CCs in selecting viruses recommended for inclusion in seasonal and pre-pandemic vaccines are shared openly and on which research scientists, public and animal health officials and the pharmaceutical industry depend. Such openness of the most up-to-date data assists in an understanding of and enhances the credibility of the WHO recommendations for the composition of these seasonal and potential-pandemic vaccines.

Furthermore, in promoting the prompt sharing of data from potential pandemic zoonotic virus infections, as well as from seasonal influenza viruses, GISAID ensures a key tenet of the WHO Pandemic Influenza Preparedness (PIP) Framework [15], highlighting the critical role it plays in mounting an effective mitigating response. GISAID's ability to facilitate efficient global collaborations, such as the Global Consortium for H5N8 and Related Influenza Viruses [16,17], is central to monitoring phylogeographic interrelationships among, for example, H5 subtype viruses in wild and domestic birds in relation to their incidence, cross-border spread and veterinary impact, and assessing risk to animal and human health [18].

Traditional public-domain archives such as GenBank, where sharing and use of data takes place anonymously, fulfil a need for an archive of largely published data; however, that conventional method of data exchange notably has not been successful in encouraging rapid sharing of important data in epidemic or (potential) pandemic situations, such as those caused by Middle East respiratory syndrome coronavirus (MERS-CoV) and Ebola viruses. While the GISAID EpiFlu database is hosted and its sustainability ensured through the commitment of the Federal Republic of Germany [19], the establishment of GISAID and development of the EpiFlu database was reliant to a large extent on philanthropy of one individual and voluntary contributions and generosity of many others, together with some initial financial provision by the US CDC and the German Max Planck Society.

That GISAID has become accepted as a pragmatic means of meeting the needs of the influenza community in part reflects the particular characteristics of influenza and the continual need for year-round monitoring of the viruses circulating worldwide, essential for the biannual vaccine recommendations and assessment of the risk posed by frequent zoonotic infections by animal influenza viruses [20]. In the meantime, calls for an equivalent mechanism to promote the timely sharing of data in other urgent epidemic settings go largely unfulfilled [21,22]. A recent publication considered whether the 'paradigm shift' in data sharing by GISAID could be applied more generally to assist in preparedness for and response to other emergent infectious threats, such as those posed by Ebola virus [21] and Zika virus [23]. Such a trusted system could complement and take full advantage of the latest advances in rapid sequencing of specimens in the laboratory and in the field, for outbreak investigation [24].

Given the crucial importance of genetic data in improving our understanding of the progress of an emergent, potentially devastating epidemic, the effectiveness of GISAID in influenza pandemic preparedness is self-evident and provides important lessons for future pandemic threats. While the genetic makeup and the necessary associated data of the different viruses are distinct requiring separate databases/compartments for unambiguous analysis, the modi operandi for sharing genetic data are generic and the GISAID mechanism could be applied to other emerging pathogens. Indeed, the wider implementation of such a data sharing mechanism should be key in concerted efforts to contain spread of disease in animals and threats to human health, in realising the concept of One Health.

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Conflict of interest

None declared.

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Research article

Significantly elevated number of human infections with H7N9 virus in Jiangsu in eastern China, October 2016 to January 2017

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Since first identified in 2013, the H7N9 virus has caused several waves of human infections in China, with a current wave including a number of patients with very severe disease. Jiangsu is one of the most impacted provinces, whereby as of 31 January 2017, the number of human infections (n = 109) in the ongoing fifth H7N9 wave has exceeded the sum of those in the four preceding ones. Ten of 13 cities in Jiangsu have been affected, and clustered infections as well as one co-infection with seasonal influenza have been observed. With a median age of 58 years and 74.3% (81/109) of patients being male, the characteristics of cases are similar to those in previous waves, however patients with H7N9 seem to have an accelerated disease progression. Preliminary case fatality remains above 30%. No significant viral mutations have been found in key functional loci. Environmental H7N9 detection rate and number of days with high risk ambient temperatures were both significantly elevated during the month of December 2016 when most human infections were reported. A number of municipal governments in Jiangsu have implemented live poultry market closures to impede viral transmission to humans. A detectable decline in human infections has been observed in these municipalities and the entire province since January 2017.

Introduction

Avian influenza viruses cause human infections continuously worldwide. Compared with other viruses, such as H5N1, H5N6, H9N2 and H7N7, the H7N9 virus is much more potent due to its better ability to cross the species barriers and infect both poultry and humans, and this has raised serious concerns for potential pandemics [1]. As of 16 January 2017, a total of 918 laboratory-confirmed cases of human infection with H7N9 virus in China have been reported to the World Health Organization (WHO) in less than four years (since March 2013) [2]. In contrast, at the same date, and for a period of over 13 years (since 2003) the number of H5N1 cases worldwide was only 856 [2].

Since its first identification in March 2013, the H7N9 virus has caused five waves of human infections in China [3]. There were 134 cases, 304 cases, 219 cases and 118 cases detected and reported in the first four waves, respectively, with a declining trend in incidence [4]. However, the fifth wave (since September 2016) surged with a steep increase in case numbers from 1 December 2016, and 106 cases were reported in December 2016 alone. As of 31 December 2016, the number of reported cases in the fifth wave was 11.4, 2.7 and 6.1 times that of the corresponding periods in the second (10 cases), third (31 cases) and fourth (16 cases) waves, respectively. Seven provinces in China have been affected, with Jiangsu being one of the most impacted [3]. Overall, the majority of reported human infections occurred in older males (median age: 57 years). The general case fatality was around 41% [4]. Here we describe the current fifth wave of human infections with H7N9 in Jiangsu province, which was characterised by a significantly elevated incidence in a wider affected area. Factors potentially contributing to the epidemic, such as meteorological factors, environmental detection rates of H7N9, and viral mutations, are also explored and discussed.

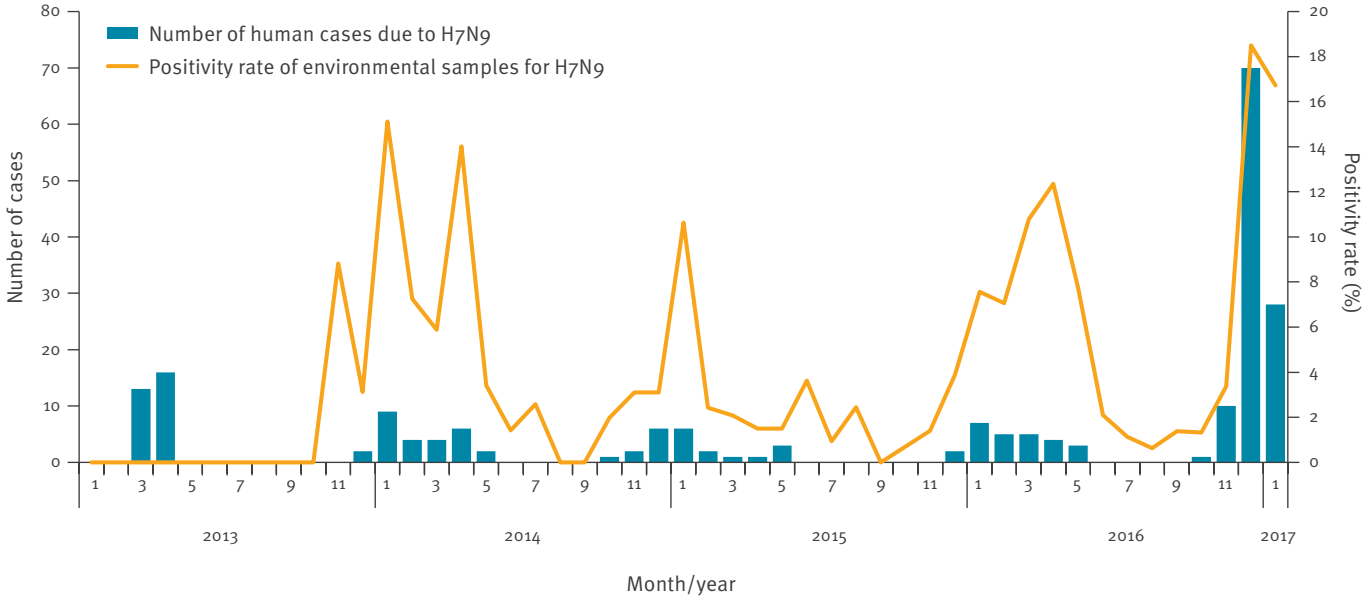
Methods

Human surveillance

In China, all laboratory-confirmed human infections with H7N9 are reported through a national system for reporting of notifiable infectious diseases [5]. In Jiangsu province, respiratory samples from suspected

Figure 1

Number of human cases due to H7N9 and positivity rate of environmental samples for H7N9 in Jiangsu province, 2013–31 January 2017 (n = 213 cases)



H7N9 patients are tested for H7N9 virus as well as common types of seasonal influenza (such as H1N1, H3N2 and B) by local municipal Centers for Disease Control and Prevention (CDC) using real-time PCR. The H7N9 avian influenza nucleotide test kits (bioPerfectus technologies, Taizhou, China) are most commonly used. The demographic, epidemiological and clinical information of patients infected with H7N9 is collected using standardised questionnaires by local Centers for Disease Control and Prevention (CDC) staff, or trained clinical doctors, and reported to Jiangsu Provincial CDC and China CDC through this system. Jiangsu Provincial CDC is responsible for checking and monitoring the reported information and takes part in the patients' investigations if necessary. According to the Diagnosis and Treatment Scheme published by the National Health and Family planning commission of China, patients with pneumonia and either respiratory failure or any other organ dysfunction are considered as severe infections.

All patients infected with H7N9 as of 31 January 2017 in Jiangsu province (n = 213) were included in this study. All the positive samples (confirmed by real-time PCR) collected from H7N9 patients were sent by local municipal CDCs to Jiangsu Provincial CDC, where the viruses were isolated and sequenced according to a previously described procedure [6].

Environmental surveillance

Aiming to predict and assess risk of human infections, a surveillance on avian influenza virus in avian-associated sites, such as poultry farms, live poultry whole sale and retail markets, has been routinely conducted year-round in Jiangsu province since October 2013. The surveillance sites cover all 13 cities of Jiangsu province. An average of three to eight sites are covered monthly in each city, where swab samples of avian faeces, cages, or drinking water of birds and poultry for sale, are collected by each municipal CDC. All the samples are tested for avian influenza using real-time PCR by municipal CDCs and then results are reported to Jiangsu Provincial CDC.

Meteorological factors

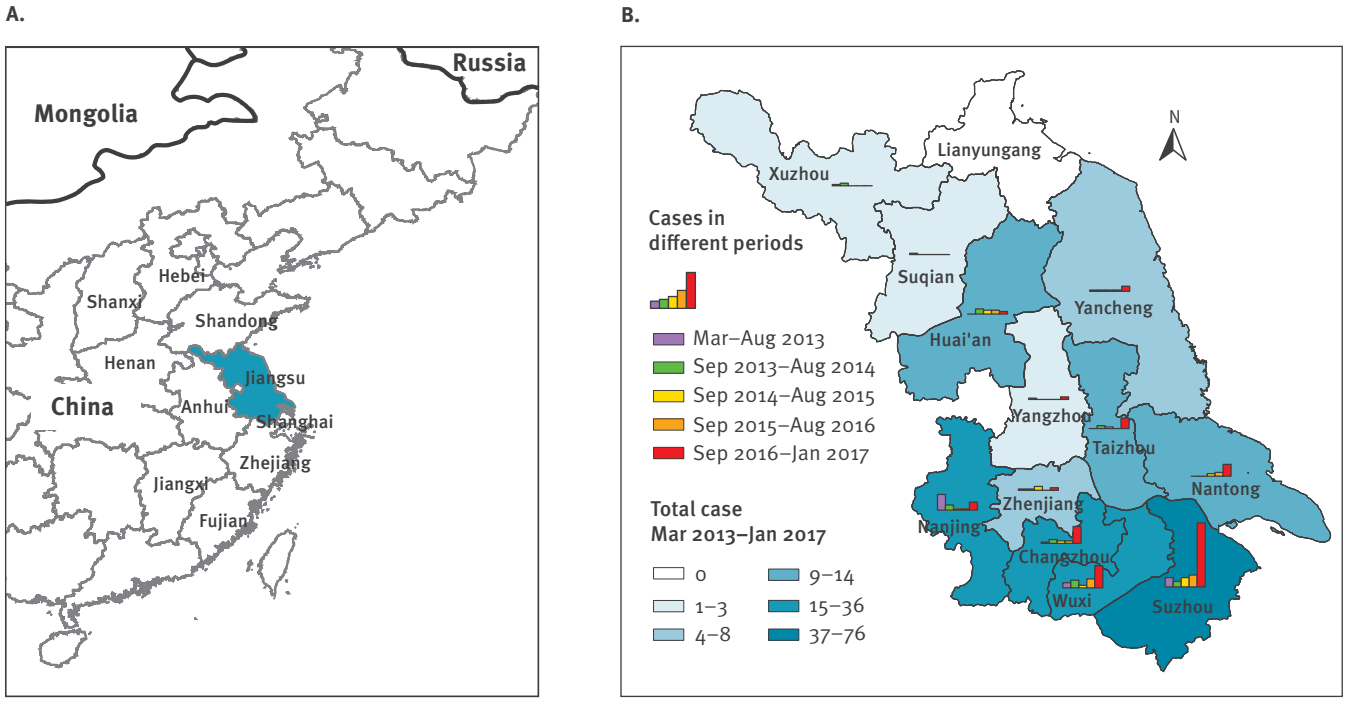
Air temperature has been previously reported to be associated with human infection with H7N9 virus, and higher risk of human infection was found when daily minimum temperatures range from ca 5 to 9 °C and when daily maximum temperatures are between ca 13 to 18 °C [7]. To investigate if air temperatures in Jiangsu might have been favourable to human infections during the fifth H7N9 wave, daily meteorological data provided by Jiangsu Provincial Meteorological Service Center were investigated.

Phylogenetic analysis

The haemagglutinin (HA) nt sequences were edited and assembled using SeqManPro (DNASTAR,Madison,WI). ClustalXv.2.1 was used for the alignment of nt sequences [8]. A phylogenetic tree of the HA1 coding nt sequences was generated by Molecular Evolutionary Genetic Analysis (MEGA) version 6.06 [9] using a neighbour-joining method with 1,000 bootstrap replicates. We acknowledge the authors, originating and submitting laboratories of the sequences from the EpiFlu Database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 1).

Figure 2

Location of Jiangsu province in eastern China, with neighbouring provinces (A) and geographical distribution of human infections with H7N9 in Jiangsu province (B), China, 2013–2017



Ethical statement

The National Health and Family Planning Commission decided that the collection of data from cases of H7N9 was part of the public health investigation of the emerging outbreak, and thus the investigation was exempt from institutional review board assessment [10]. The dataset was anonymised in the national reporting system except for individuals with special access, and was anonymised before data analyses.

Statistical analysis

Median and interquartile ranges (IQRs) were calculated for continuous variables and absolute numbers and proportions for categorical variables. Selected demographic, epidemiological and clinical characteristics of H7N9 patients were compared among five epidemic waves (March to April in 2013, December 2013 to May 2014, October 2014 to May 2015, December 2015 to May 2016 and October 2016 to 31 January 2017). Pearson chi-squared test was used for comparing proportions and continuity correction or Fisher's exact test was used if appropriate. Kruskal–Wallis H test was used for comparing medians among multiple groups. Statistical analyses were conducted using R version 3.0.2 and statistical significance level was set at ≤ 0.05. The hierarchical colour map was produced with ArcGIS software version 10.0 (ESRI, Redlands, CA, US) to state the spatial patterns of human infections with H7N9. All cases with missing data on a certain characteristic were excluded when this characteristic was analysed. Information of total and missing data for each studied variable is shown in detail in Table 2.

Results

Human infections

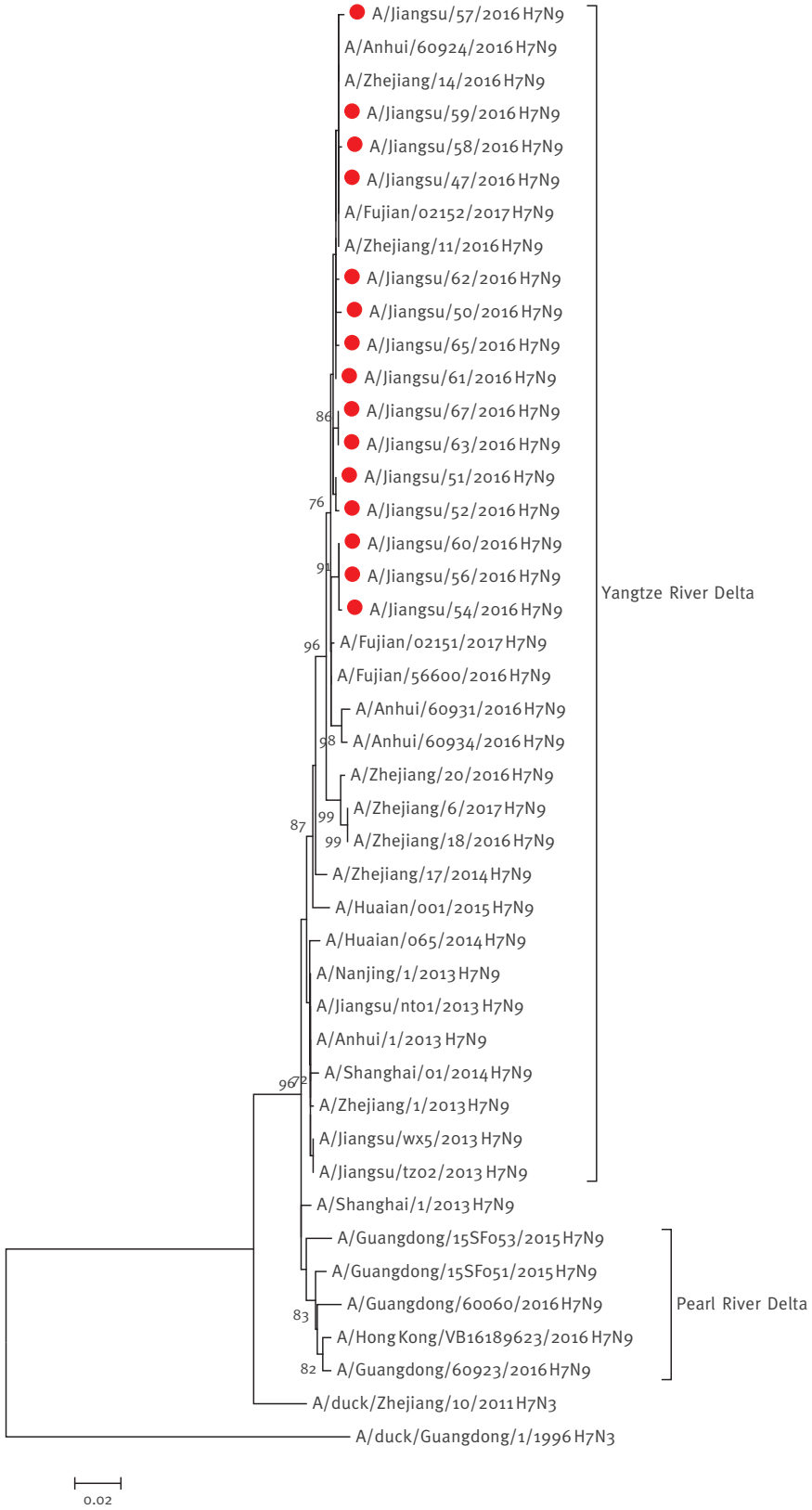
Jiangsu province is now experiencing the fifth wave of human infections with H7N9. As of 31 January 2017, the cumulated number of cases in the current wave is 109 (since October 2016), which is overwhelmingly higher than in each of the previous four waves (29, 27, 22 and 26, respectively). The peak monthly incidence in the fifth epidemic is also higher than in the previous waves (70 vs 16, 9, 6 and 7) (Figure 1).

Among recorded cases, 36 died. During the same period, a total of 305 patients were reported nationally, with 98 deaths. Patients of Jiangsu accounted for 35.7% (109/305) of those in the entire country, and this proportion was higher than in previous waves (8.9%–22.0%, $p<0.0001$). The preliminary case fatality rate, as there were 36 patients still hospitalised at the time of this analysis, was 33.0% (36/109) in Jiangsu, which is slightly higher than that of the rest of China (31.6%, 62/196). Ten of 13 cities in Jiangsu province reported human infections during the fifth wave, which is also more than previously (7–9 cities). Most of the cases were reported from Suzhou (49/109, 45.0%), Wuxi (17/109, 15.6%) and Changzhou (13/109, 11.9%). These three cities are adjacent and all located in southern Jiangsu province (Figure 2).

In the fifth wave in Jiangsu province, male patients accounted for 74.3% (n=81) of the reported 109 patients, and the overall median age was 58 years.

Figure 3

Phylogenetic analysis of haemagglutinin (HA) sequences of H7N9 viruses derived from patients reported from October to December 2016 in Jiangsu province and H7N9 and H7N3 HA sequences identified earlier in China, 1996–2016



The phylogenetic tree of the HA1 coding nt sequences was generated by Molecular Evolutionary Genetic Analysis (MEGA) version 6.06 by neighbour-joining method with 1,000 bootstrap replicates; only bootstrap values over 70 are shown. Red circles indicate H7N9 viruses isolated from Jiangsu province, 2016.

The proportion of severe infections as well as having poultry or poultry market exposure history remained high (93.0% and 70.8%, respectively) as in the previous waves. No significant differences were observed in patients' demographic characteristics (age, sex and BMI), poultry or live poultry market exposure history, and proportion of severe infections and deaths between this wave and previous waves. However, patients' disease progression seemed to be accelerating during late waves. The median time intervals from onset of disease to intensive care unit (ICU) admission were around 7 days during the latest three waves, which was shorter than that of the first and the second wave (10 and 9 days, $p = 0.048$). The median time interval from onset of disease to death was 13.5 days in the current wave, which was significantly shorter than that of previous four waves (15–28 days, $p < 0.0001$) (Table 3).

Three clustered human infections with H7N9 virus have been reported in previous waves in Jiangsu province, one in the first wave [11] and another two in the fourth (data not shown). During the fifth wave, another cluster of probable human-to-human transmission with laboratory evidence (viral sequence similarity > 99.99%) was reported in Suzhou city. The index case, a person aged in their mid-60s, visited live poultry markets regularly before getting ill. Three days after this case's onset of illness, a relative in their late 30s, took care of the index patient while in hospital, for a duration of three days, without taking any precautions. The relative had contact with sputum and the body of the index patient during this period and developed symptoms six days later. Two days after symptom onset, this secondary case was also admitted to hospital. Both patients were severely ill with diagnosis of pneumonia. The secondary patient recovered and was discharged from the hospital 25 days after their illness started, but the index patient died 17 days after disease onset.

Seasonal influenza activity peaked with H3N2 being the predominant subtype during the same period (December 2016) in Jiangsu province. Similar to 2013, when we reported a patient co-infected with H7N9 and H3N2 virus [12], another such patient was identified in 2016 using real-time PCR. The patient, aged in their late 50s lived in Suzhou, without chronic medical conditions and without seasonal influenza vaccination history. Three days prior to symptom onset, this person had visited a live poultry market together with their spouse and bought two chickens and one goose, without direct contact with these live poultry. Seven days after disease onset, the patient was admitted to hospital with severe pneumonia and died 13 days later. The spouse did not develop any symptoms.

Environmental virus detection

The environmental H7N9 virus detection rate was found significantly elevated in Jiangsu during this wave as well ($p < 0.0001$). It peaked at 18.5% (149/805) in December 2016 when most of the human infections of the ongoing fifth wave were reported, while the rate was only 3.1% (2/65), 3.1% (11/359) and 3.9% (22/561) during the same period of 2013, 2014 and 2015, respectively (Figure 1). In addition, an increased detection of H7N9 virus in environmental samples collected during the summer was noted. The virus was not detected in August and September in 2014 and in September in 2015, but was detected in both August and September in 2016 (Figure 1).

Meteorological impact

We found that there were nine days with high risk minimum temperature (provincial mean) in December 2016 when most of the human infections were reported, which was significantly more than that of the same period in past three years (0, 0 and 4 in 2013, 2014 and 2015 respectively, $p < 0.0001$). The number of days with high risk maximum temperature (provincial mean) in December 2016 was also significantly more than in the past 3 years (10 vs 5, 2 and 3 days respectively, $p = 0.028$). In November 2016 however, while the numbers of 'high risk' days were similar to that of December 2016 (10 days vs 9 days for minimum temperature, 12 days vs 10 days for maximum temperature), the environmental H7N9 virus detection rate was remarkably lower than that of December (3.38% vs 18.50%), as was also the rate of human infections (9 in November vs 70 in December). The daily temperature data of January 2017 are unavailable at present.

Viral analyses

Whole genomes of fifteen H7N9 strains, which were isolated from patients reported during this wave (1 in October, 2 in November and 12 in December 2016) in Jiangsu province, were sequenced and analysed. All the strains were from severely affected cities, that is, Suzhou ($n = 10$ total cases), Changzhou ($n = 3$) and Wuxi ($n = 2$). A phylogenetic tree of the HA genes of these strains was produced together with viruses isolated previously. The results showed that viruses isolated during the current wave shared the same ancestor as earlier viruses from 2013 to 2015, but clustered in an independent clade (Figure 3), which suggested that H7N9 virus is continuously evolving.

Genetic characteristic analysis indicated that significant mutations have not occurred in these H7N9 viruses. No substitutions were observed in two positions (G186V and Q226L) located in the receptor-binding sites of HA, indicating that the virus retains the ability to bind with both avian α2,3-sialic acid and human α2,6-sialic acid receptors. The R294K mutation in the neuraminidase (NA) protein, which is believed to confer resistance to NA inhibitors [13] was not observed in these isolates. Furthermore, the substitution of E627K in polymerase basic protein 2 (PB2) protein was not observed either, which indicates that viruses could not efficiently replicate in human [14].

Table 1

Origin of the haemagglutinin sequences of influenza A(H7N9) used for the phylogenetic analysis in this study

Segment ID	Segment	Country	Collection date	Isolate name	Originating Laboratory	Submitting Laboratory	Authors
EPI872958	HA	Hong Kong SAR	2016-Dec-28	A/Hong Kong/VB16189623/2016	Public Health Laboratory Services Branch, Centre for Health Protection	Public Health Laboratory Services Branch, Centre for Health Protection	Mak,G.C.; Lo,J.Y.C.
EPI887844	HA	China	2016-Dec-09	A/Guangdong/60060/2016	Guandong Centers for Disease Control	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887836	HA	China	2016-Dec-28	A/Guangdong/60923/2016	Guandong Centers for Disease Control	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887772	HA	China	2016-Dec-21	A/Zhejiang/11/2016	Zhejiang Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887748	HA	China	2016-Dec-23	A/Zhejiang/14/2016	Zhejiang Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887692	HA	China	2016-Dec-27	A/Zhejiang/18/2016	Zhejiang Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887668	HA	China	2016-Dec-30	A/Zhejiang/20/2016	Zhejiang Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887652	HA	China	2017-Jan-05	A/Zhejiang/6/2017	Zhejiang Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI888052	HA	China	2016-Dec-19	A/Anhui/60934/2016	Anhui Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI888036	HA	China	2016-Dec-13	A/Anhui/60931/2016	Anhui Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887996	HA	China	2016-Dec-22	A/Anhui/60924/2016	Anhui Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887852	HA	China	2016-Nov-20	A/Fujian/56600/2016	Fujian Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887620	HA	China	2017-Jan-06	A/Fujian/02152/2017	Fujian Provincial Center for Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong
EPI887612	HA	China	2017-Jan-01	A/Fujian/02151/2017	Fujian Provincial Center For Disease Control and Prevention	WHO Chinese National Influenza Center	Wang,Dayan;Zhou,Shumei;Li,Xiyan;Li,Xiaodan;Zhang,Ye;Bo,Hong;Shu,Yuelong

HA: haemagglutinin; WHO: World Health Organization.

Table 2

Number of missing/total data of selected characteristics of H7N9 patients in Jiangsu province, March 2013–January 2017

Selected characteristics	Proportion of patients among the total missing information on a characteristic				
	Wave 1 (Mar to Apr 2013)	Wave 2 (Dec 2013 to May 2014)	Wave 3 (Oct 2014 to May 2015)	Wave 4 (Dec 2015 to May 2016)	Wave 5 (Oct 2016 to 31 Jan 2017)
Age	0/29	0/27	0/22	0/26	0/109
Sex	0/29	0/27	0/22	0/26	0/109
BMI	1/29	0/27	2/22	1/26	9/109
Poultry or live poultry market exposure	2/29	0/27	2/22	0/26	15/109
Severe infection	2/29	0/27	2/22	0/26	9/109
ICU admission	2/29	0/27	2/22	1/26	33/109
Death	0/29	2/27	2/22	1/26	0/109
Number of days from onset of disease to ICU admission	0/21	0/ 20	0/17	0/20	0/65
Number of days from onset of disease to death	0/10	0/13	0 /13	0/11	0/36

BMI: body mass index; ICU: intensive care unit.

Discussion

Jiangsu province is now experiencing the fifth wave of human infections with H7N9, with a significantly elevated number of cases. Visiting live poultry markets is the main risk factor for H7N9 infection for the public, due to poultry contact in this setting or environmental contamination [15,16]. The high H7N9 virus detection rate in these sites may directly contribute to the elevated human H7N9 infections. Therefore, live poultry market closures have been suggested as an effective method to control such infections [17,18]. Accordingly, several municipal governments in Jiangsu province, including Suzhou, Wuxi and Changzhou, have implemented temporary comprehensive live poultry market closures since December 2016. Subsequently, a significant decline in human infections has been observed in these cities/municipalities, as well as province-wide since January 2017 (Figure 1). The decrease was even more evident in February (data not shown).

Many meteorological factors, such as temperature, relative humidity [7,19], specific humidity [20] and solar radiation [21], have been reported to influence influenza activity. As for H7N9, both daily minimum and daily maximum temperatures have been reported to contribute significantly to human infection, but not relative humidity [22]. Other meteorological factors have not been reported. The overall impact of ambient temperature on human infection rates with H7N9 may nevertheless also depend on the underlying level of environmental H7N9 virus contamination, as exemplified by the results in November 2016, when although temperatures appeared to be permissive to human infection, low rates were observed, coinciding with low rates of environmental contamination. The environmental H7N9 contamination rate could be influenced by multiple factors, such as H7N9 virus infection rate of poultry for sale, and the hygiene level of the live poultry market. The interaction and correlation between temperature and other factors and their impact on human infections need to be investigated further in future studies.

Antivirals such as oseltamivir were administrated to almost all of the H7N9 patients in recent years in Jiangsu province. Furthermore, the time interval from onset of disease to antiviral administration is becoming shorter due to promoted sensitivity of clinicians. Clinicians also gained experiences in treatment, such as the rational use of ventilators and extracorporeal membrane oxygenation (ECMO). All of these measures are beneficial for the patients' clinical outcome. Nevertheless, an accelerated disease progression of H7N9 patients during latest waves was still observed, which suggests that the viral pathogenicity might have become stronger. In addition, the increased detection rate of H7N9 in environmental samples suggests that the virus might become more resistant to high ambient temperature. Although no significant mutations were observed in key functional loci of the isolates from the current wave in our preliminary analyses, further work still needs to be conducted in detail. For instance, changes in the length of the neuraminidase stalk region might impact virulence [23] and residues 41V and/or 210D in the nucleoprotein (NP) protein could enhance polymerase activities and potential replication at low temperature [24].

The pandemic potential of the H7N9 virus needs to be closely watched. In humans, co-infection of this virus with seasonal influenza might provide reassortment opportunities for the emergence of a new pandemic virus. In addition, the continuous mutation and reassortment of H7N9 with other avian influenza viruses lately resulted in the identification of H7N9 isolates with characteristics of high pathogenicity to poultry, which was concerning for the poultry industry [25]. There is also a risk that H7N9 might acquire better ability of spreading from poultry to ducks and wild birds, and thus be disseminated worldwide, threatening humans in a much wider geographical range [26-28]. Therefore, it is critical to control the transmission of H7N9 virus in poultry to lower these risks.

Table 3

Comparisons of selected characteristics of H7N9 patients among five epidemic waves in Jiangsu province of eastern China, 2013–31 January 2017

Selected characteristics	Wave 1 (Mar to Apr 2013) n = 29	Wave 2 (Dec 2013 to May 2014) n = 27	Wave 3 (Oct 2014 to May 2015) n = 22	Wave 4 (Dec 2015 to May 2016) n = 26	Wave 5 (Oct 2016 to 31 Jan 2017) n = 109	P[a]
Age, years, median, (IQRs)	54 (35–70)	53 (42–66)	57 (50–68)	53 (41–63)	58 (46–66)	0.482
Proportion of male cases	21/29	20/27	14/22	18/26	81/109	0.860
BMI, median (IQRs)	22.94 (22.17–24.16)	23.44 (21.97–26.12)	23.85 (22.49–26.55)	24.01 (21.23–26.37)	24.22 (22.49–26.12)	0.472
Proportion of cases with poultry or live poultry market exposure	14/20	20/27	15/20	20/26	63/89	0.979
Proportion of severe infections	23/28	26/27	18/20	24/26	93/100	0.398
Proportions of ICU admission	21/27	20/27	17/20	20/25	65/76	0.665
Proportion of deaths	10/29	13/27	13/22	10/26	36/109[b]	0.586
Median number of days (IQR) from onset of disease to ICU admission	10 (7–14)	9 (8–11)	7 (6–9)	7.5 (6–10)	7 (6–10)	0.048
Medium number of days (IQR) from onset of disease to death	28 (20–45)	24 (20–38)	15 (13–23)	22.5 (13–42)	13.5 (8–20.5)	< 0.0001

BMI: body mass index; IQRs: interquartile ranges.

[a] Pearson chi-squared test was used for comparing proportions and Kruskal–Wallis H test was used for comparing medians.

[b] There were 36 patients still in hospital at the time of the study.

To avoid the possibility of further adaption to human of this virus, early identification of human infections with H7N9 and early administration of neuraminidase inhibitors are critically needed. At present, the median time intervals from onset of disease to first medical consultation and from onset of disease to administration of neuraminidase inhibitors are two and six days, respectively. Efforts implementing effective rapid diagnostic kits in primary medical facilities, such as community clinics, could further promote the timeliness of diagnosis and antiviral therapy, as nearly half of the H7N9 patients first seek medical services in these facilities.

Until now, older males still account for most of the H7N9 patients. An overwhelming majority of the reported patients were severely infected and the overall case fatality remained above 30%. Live bird markets are the most common sites for the public to contact birds or bird materials which might carry H7N9 virus. With the continuous closures of live bird markets, the case number is expected to keep decreasing. In addition, the upcoming warmer weather would also deter the transmission of H7N9. However, we should be alert that H7N9 cases might occur in areas where live bird market closures are not implemented, also because live poultry from places affected by H7N9 and with market closures, may be transferred to these areas. A full investigation of the current wave of human infections with H7N9 virus is still ongoing. This study presents timely preliminary results, including possible causes, which could help researchers in further detailed analyses.

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Conflict of interest

None declared.

Authors' contributions

C.B., X.H., L.C. and J.Z. conceived of the study and participated in the design of the study. X.Q., H.Y. and Y.X. performed the laboratory work and analyses. L.C., H.H, W.L., Q.D., K.X and W.M. carried out the epidemiological investigations and analyses. X.H. drafted the manuscript and all authors read and approved the final manuscript.

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Research article

Risk factors for MERS coronavirus infection in dromedary camels in Burkina Faso, Ethiopia, and Morocco, 2015

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Understanding Middle East respiratory syndrome coronavirus (MERS-CoV) transmission in dromedary camels is important, as they consitute a source of zoonotic infection to humans. To identify risk factors for MERS-CoV infection in camels bred in diverse conditions in Burkina Faso, Ethiopia and Morocco, blood samples and nasal swabs were sampled in February–March 2015. A relatively high MERS-CoV RNA rate was detected in Ethiopia (up to 15.7%; 95% confidence interval (CI): 8.2–28.0), followed by Burkina Faso (up to 12.2%; 95% CI: 7–20.4) and Morocco (up to 7.6%; 95% CI: 1.9–26.1). The RNA detection rate was higher in camels bred for milk or meat than in camels for transport (p = 0.01) as well as in younger camels (p = 0.06). High seropositivity rates (up to 100%; 95% CI: 100–100 and 99.4%; 95% CI: 95.4–99.9) were found in Morocco and Ethiopia, followed by Burkina Faso (up to 84.6%; 95% CI: 77.2–89.9). Seropositivity rates were higher in large/medium herds (≥51 camels) than small herds (p = 0.061), in camels raised for meat or milk than for transport (p = 0.01), and in nomadic or sedentary herds than in herds with a mix of these lifestyles (p < 0.005).

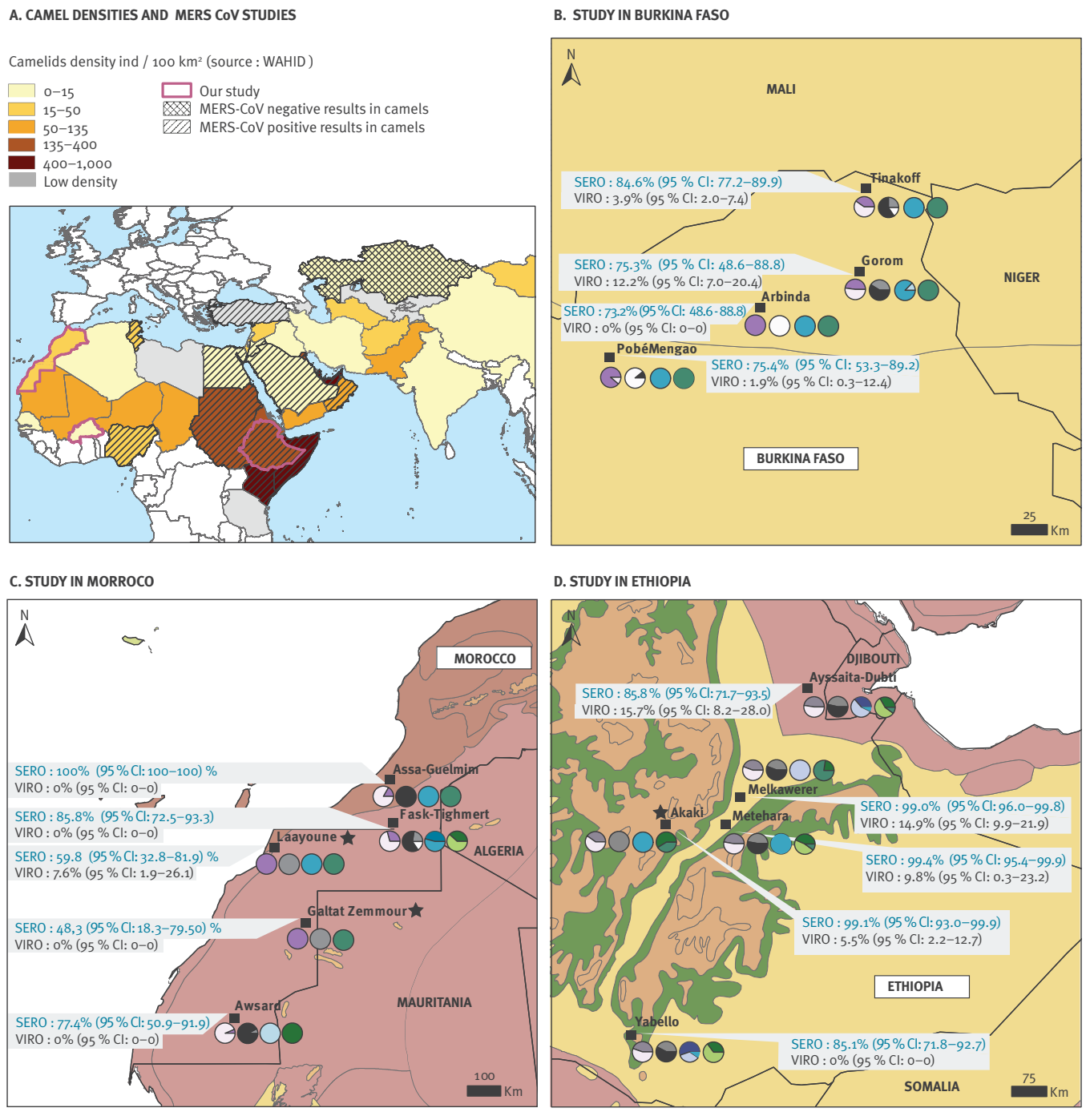
Introduction

In September 2012, a novel coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), was identified from a patient with a fatal viral pneumonia in Saudi Arabia. This coronavirus is genetically related, but not identical, to the severe acute respiratory syndrome (SARS) coronavirus which emerged in southern China in 2002 [1]. As of 21 March 2017, 1,917 human cases have been reported to the World Health Organization (WHO) with at least 684 deaths [2]. Most zoonotic infections have occurred in the Arabian Peninsula, particularly in Saudi Arabia, although nosocomial outbreaks arising from travellers coming from the Arabian Peninsula have been reported in Africa, Asia, Europe and North America. For example, between May and June 2015, 186 human infections in South Korea arose from one returning traveller [3], highlighting the cause for global public health concern.

Human disease ranges from mild or asymptomatic infection to a fulminant viral pneumonia progressing to severe respiratory failure and death. Dromedary camels are strongly suspected to be the source of human infections [4]. It is believed that humans can get infected via direct contact with mucous membranes of infected camels [5,6] or by consuming unpasteurised camel milk [7]. However, the virus has not been detected in camel urine [8] or in raw camel meat [9]. Secondary infections in humans are reported, especially within nosocomial settings [10,11] or to a smaller extent, within households [12], suggesting that human-to-human transmission may become efficient enough to trigger outbreaks beyond the current epicentre in the Middle East. The WHO has identified MERS-CoV as one of the pathogens of greatest concern for global public health for which few or no medical countermeasures exist [13]. To date there are no vaccines or antivirals available for MERS-CoV in humans [14]. Camel vaccines have given promising results with the use of a vaccinia Ankara (MVA) vectored vaccine [15].

Figure 1

A. Camel densities in Africa, Middle East, and Asia with areas with prior serological evidence for MERS-CoV infection in camels, and B–D. sampling sites of this study, with serological and virological MERS-CoV detection rates in Burkina Faso, Ethiopia and Morocco, February–March 2015



Ind: individuals; MERS-CoV: Middle East respiratory syndrome coronavirus; SERO: serological detection rate; VIRO: virological RNA detection rate.

A. Update of the MERS-CoV negative and positive serological results in camels published in literature (hatched areas) and the countries selected for the study i.e. Burkina Faso, Ethiopia and Morocco (with thick borders).

B, C, D. Regions sampled by country with their farming specificities (different pie charts according to camel sex, function, lifestyle and herd size), the local ecoregions, and the estimated seropositivity and viral RNA detection rates by region from the cross-sectional survey done in February–March 2015.

MERS-CoV only causes mild respiratory symptoms in camels and it is consequently not easily recognised and difficult to diagnose clinically. High levels of seropositivity and virus detection rates have been observed in dromedary camels in the Arabian Peninsula [16,17]. MERS coronaviruses detected in camels are genetically very similar or identical to those infecting humans [18]. MERS-CoV antibodies have also been detected in dromedary camel populations of many countries outside the Arabian Peninsula. Serological studies in Africa indicate high seropositivity rates and the testing of retrospectively collected serum samples provide evidence that this virus has been infecting camels in East Africa since as early as 1983 [19]. More recent specimens collected between 2009 and 2013 show high rates of detection of MERS-CoV antibodies in camels in Egypt, Ethiopia, Kenya, Nigeria, Sudan and Tunisia and also in the Canary Islands [20,21].

Surprisingly, the only indication of locally acquired primary zoonotic human infections outside the Arabian Peninsula is the recent detection of antibodies against MERS-CoV in autochthonous livestock handlers in Kenya between 2013 and 2014 [22]. Possible reasons for the absence of reports of MERS-CoV infections in humans in Africa may include (i) underdiagnosis in humans due to a possible lack of awareness, lack of viral diagnostic capacity and weak healthcare systems, (ii) differences in virus strains or in camel breeds resulting in low infectiousness towards humans, (iii) differences in cultural practices in interaction between humans and dromedary camels, or any combination of these. Research recommendations from workshops on MERS-CoV in Doha April and Cairo May 2015, organised by the Food and Agriculture Organisation (FAO), the Organisation of the United Nations for Animal Health (OIE) and the WHO identified the apparent absence of human MERS-CoV infections in Africa despite intense virus circulation among dromedaries as a key research question [23]. In order to address this question, it is important to understand the ecological and farming husbandry factors that may promote the likelihood of MERS-CoV infection in camels in Africa.

We report a descriptive serological and virological survey of MERS-CoV from west to east across the African continent, which was conducted by sampling camels in Burkina Faso, Ethiopia and Moroco. Sampling was designed so as to also assess the influence of the herd size, camel function (raised for milk, meat or transport) and lifestyle (either nomadic, sedentary or a mix of the two lifestyles) on likelihood of MERS-CoV infection.

Methods

Study sites and camel farming

Nomadic, sedentary, mixed lifestyles and extensive, semi-extensive and intensive camel breeding systems occur in African ecosystems. Extensive system/nomadic lifestyle are characterised by the use of natural resources, low inputs, and herd mobility [24]. However, camel husbandry practices and the use of camels have changed in the last five decades in the following ways: (i) increasing camel populations in settled livestock farming systems, (ii) use of camels in agriculture-related work, (iii) camel trade being more closely market integrated and (iv) increasing importance of camels for the sustainability and resilience of farms which traditionally relied on cattle [25]. These changing camel herding practices lead to sedentary or mixed lifestyles with intensive or semi-intensive camel production systems (milk, meat, skin etc.) [26]. Usually, camel calves are suckled by their mother during the first year of life. Camels are considered as young and sexually immature until 2–4 years-old. Males represent 20 to 40% of the herd [27]. Adult males are separated from females and young camels in non-extensive systems because of their aggressiveness associated with sexual behaviour. In extensive systems, the male is let with non-lactating females for reproduction only and during the rutting season. The contacts of adult males with young (less than 4 years-old) camels is not common.

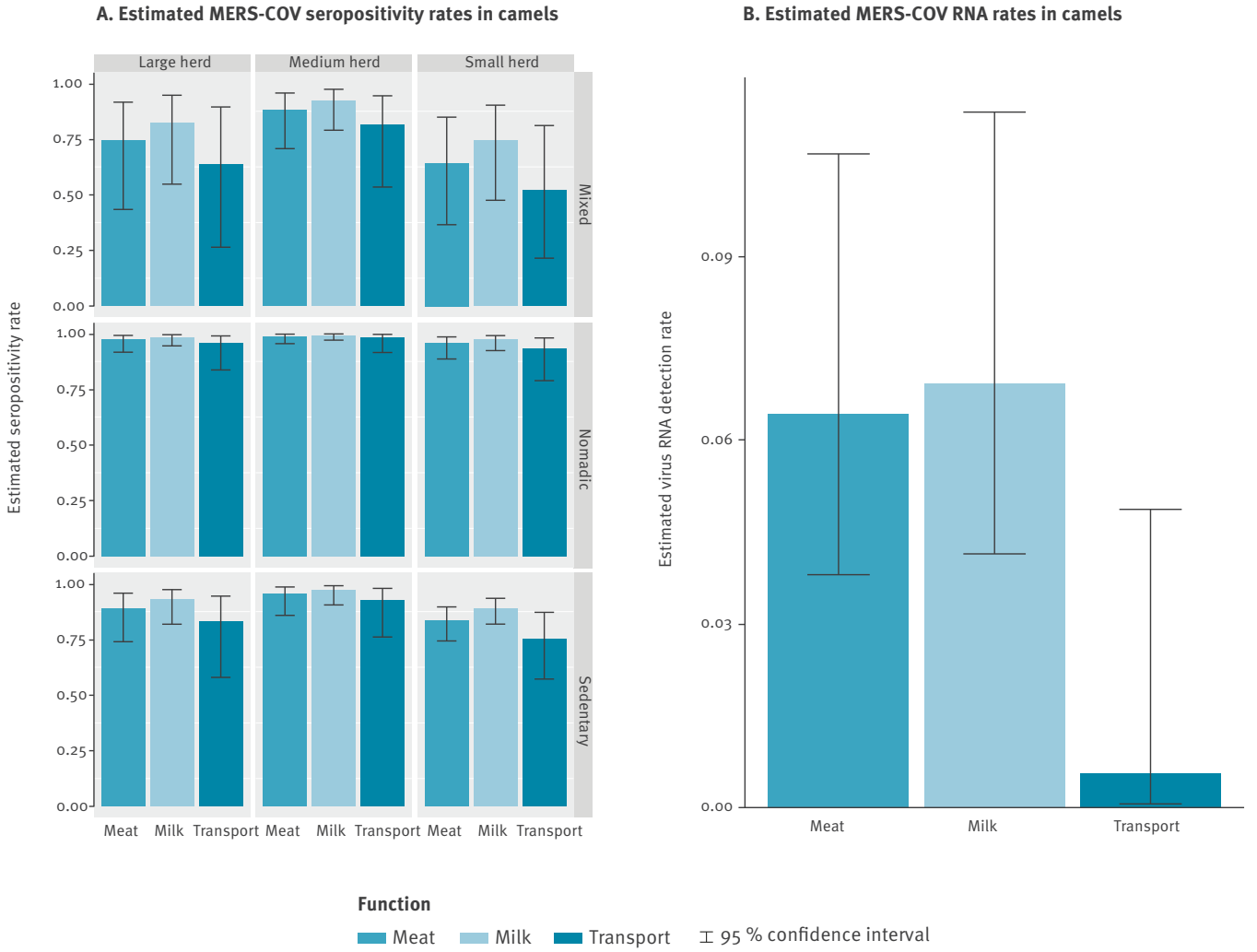
Camel density increases from North to East Africa through the Sahelian strip with the highest densities recorded in the Greater Horn of Africa which harbours 60% of the world population [9] (i.e. with 400–1,000 individuals/100 km2 in Kenya and Somalia for instance; Figure 1A). Our sampling design covers Burkina Faso, Ethiopia and Morocco and a diversity of farming systems in different contexts (Figure 1B–D). Camel density is estimated at 0.07 individuals/km2 with 18,374 camels in Bukina Faso, 1.99 individuals/km2 with 2,245,581 camels in Ethiopia, and 0.44 individuals/km2 with 197,550 camels in Morocco [27]. Camel population densities are available at the country level only. However as camels are dependant on specific ecosystems which are mainly deserts or tropical and subtropical grasslands, savannas and shrublands [28] (Figure 1B–D), they are not distributed homogeneously in each country. Unfortunately, statistics on regional densities are not available.

Field work

The field work was done between February and March 2015 in collaboration with the animal health institutes from Burkina Faso (Laboratoire de Biologie et Santé Animales - INERA-CNRST), Ethiopia (National Veterinary Institute) and Morocco (Institut Agronomique et Vétérinaire Hassan II). Cross-sectional studies were carried out simultaneously in the three countries. Blood (for serological analyses) and nasal swabs (for virological analyses) were collected from camels. The swabs were placed in virus medium transport. The blood samples were allowed to clot at room temperature and the serum extracted with a pipette. Swabs and sera were placed in cool box with ice packs if a –80 °C freezer was reachable in 48 h or otherwise frozen in a liquid nitrogen tank. On arrival at the national laboratory, all the samples were stored in a –80 °C freezer before their shipment to the international reference laboratory at the University of Hong Kong, for MERS-CoV serological

Figure 2

MERS-CoV seropositivity and viral RNA detection rates estimated by modelling according to significant risk factors, Burkina Faso, Ethiopia and Morocco, February–March 2015



MERS-CoV: Middle East respiratory syndrome coronavirus.

and virological analyses. Questionnaires to ascertain camel habitats, environment and farming practices were administered to the farmers by veterinarians after specimen collection.

Camels raised for three distinct functions (milk, meat or transport) were sampled. Herd size was classified into three categories (small with ≤ 50 camels, medium with 51 to 150 camels and large with 151 to 300 camels). Samples were collected at two types of sites, with the majority taken at farms (1,301 samples from 80 herds) and some at abattoirs (199 samples from 6 herds) in Ethiopia and Morocco (Table 1).

Sampled camels were classified into one of three distinct lifestyles (nomadic, sedentary or a mix of nomadic and sedentary). The mixed lifestyle is characterised by a seasonal spatial movement of less than 100 km for accessing new ressources while the nomadic lifestlyle was defined as travelling throughout the year over distances up to hundreds of kilometers.

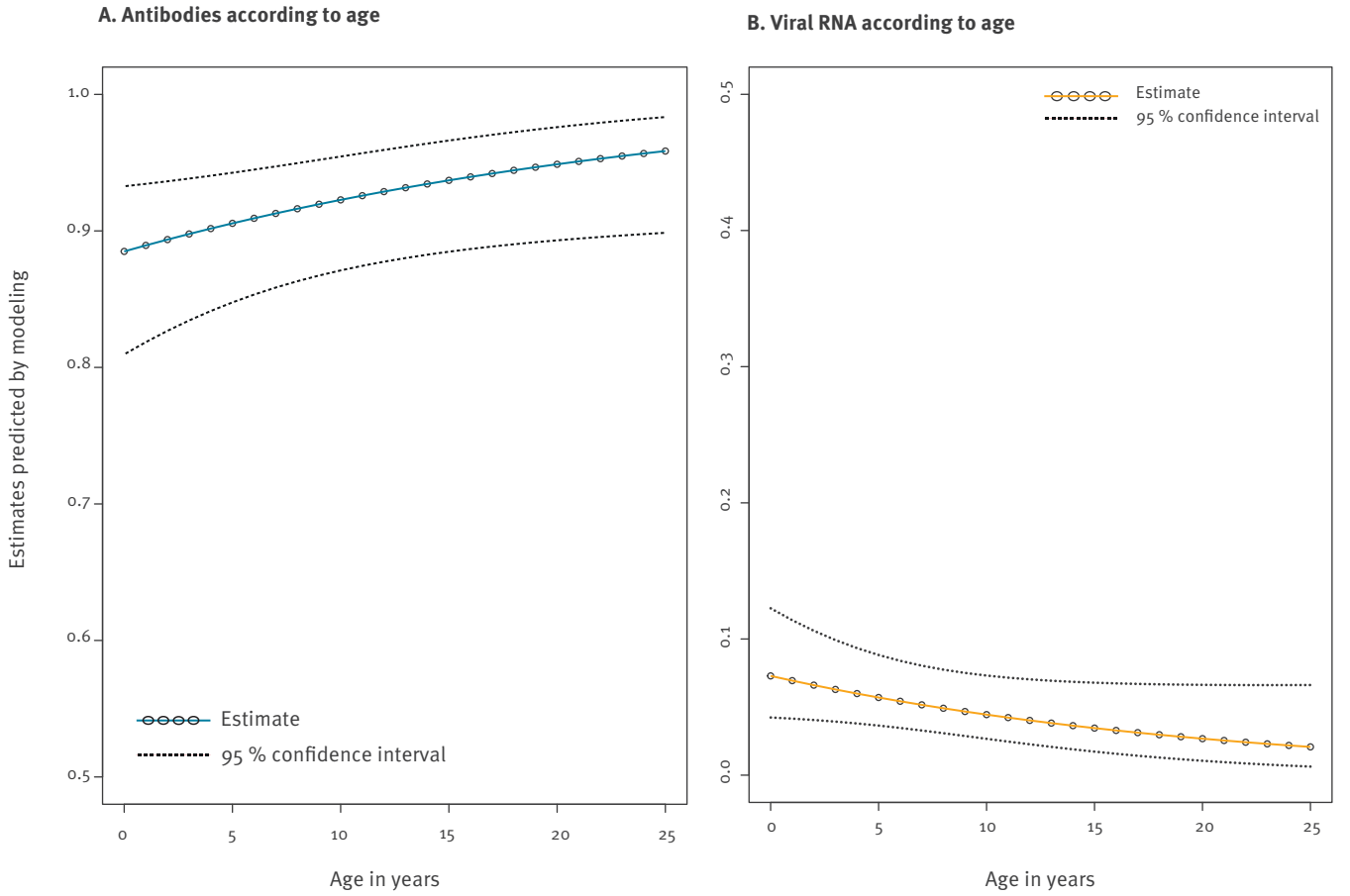
Each region has specifities in term of farming practices (Figure 1B–D). For example in Morocco, camels bred for meat are mainly young males in small herds and are sent to the abattoir (i.e. Laayoun) while camels bred for milk are females living in large nomadic herds (i.e. Awsard) (see Figure 1C for the specificities by region covered in the study).

Biological analyses

Specimens were shipped on dry ice to the University of Hong Kong. Serum samples were tested for MERS-CoV antibodies at a screening dilution of 1:20 using an extensively validated MERS-CoV (strain EMC) spike pseudoparticle neutralisation test [29]. Selected positive sera were confirmed using microneutralisation tests in biosafety level (BSL)3 containment [30]. Total

Figure 3

MERS-CoV seropositivity (antibodies) and viral RNA detection rates in camels estimated by modelling according to age, Burkina Faso, Ethiopia, Morocco, February–March 2015



MERS-CoV: Middle East respiratory syndrome coronavirus.

nucleic acid was extracted from swab samples using the EasyMag (Biomerieux) system and tested for the presence of MERS-CoV RNA using the upstream of the envelope gene (UpE) reverse transcription-quantitative PCR (RT-qPCR) hydrolysis probe assay. All positive specimens were confirmed by a second RT-qPCR assay targeting the open reading frame (ORF) 1a region of the genome as previously described [18].

Statistical models for depicting serological and virological status according to geography and risk factors

Generalised linear mixed models (GLMM), with binomial error structures, were used to depict variations in serological and virological status according to individual characteristics (sex and age), spatial localisation (country and regions) and farming practices (camel's function, herd size, sampling place and lifestyle). The results from the abattoirs were not included in the risk factor modelling due to the difficulty to get reliable information on the farms where the animals were raised. However, data from the abattoir were included for the statistical modelling of geographical variations of serological and virological rates. Indeed, the presence of an abattoir in a region may strongly influence the likelihood of infection in that region. In the statistical models, the dependent variable was binary: the serological and virological status of an individual was designated either positive or negative according to the result of the tests presented above. Because individuals were aggregated in herds, independence of statistical units was questionable. Herd random effects were thus included in the models. Goodness of fit was assessed through the Pearson overdispersion test [31]. Selection among models including different combinations of the explanatory variables was performed using Akaike information criterion [31,32].

As some of explanatory variables may be collinear, two-by-two comparisons of explanatory variables were used to assess possible confounding influences. Cramer's V (CrV) test was used for categorical variables and R2 obtained from linear models for continuous variable. When the statistic is close to 1 for R2, or larger than 0.4 for CrV test, the two explanatory variables are considered as collinear and were not be used in the

Table 1

Location, number[a] and characteristics of camels sampled for a cross-sectional serological and virological surey on MERS-CoV, Burkina Faso, Ethiopia and Morocco February–March 2015 (n=1,500 camels)

Country	Region	Tot Inds.	Inds./ herd	Sex		Function			Herd size[b]			Lifestyle			Type	
				Female	Male	Meat	Milk	Transport	Large	Medium	Small	Mixed	Nomadic	Sedentary	Abattoir	Farm
Burkina Faso	Gorom	127	12	66	61	52	74	1	0	0	127	0	16	111	0	127
	Tinakoff	289	10	172	117	73	171	45	0	0	289	0	0	289	0	289
	Arbinda	47	24	0	47	0	0	47	0	0	47	0	0	47	0	47
	PobéMengao	62	31	7	55	0	7	55	0	0	62	0	0	62	0	62
Ethiopia	Akaki[c]	100	25	60	40	100	0	0	59	0	41	0	0	100	100	0
	Ayssaita-Dubti	99	20	52	47	46	53	0	33	56	10	36	56	7	0	99
	Melkawerer	199	22	111	88	88	111	0	45	0	154	0	199	0	0	199
	Metehara	140	23	74	66	66	74	0	61	65	14	0	0	140	0	140
	Yabello	94	24	52	42	40	54	0	33	61	0	52	33	9	0	94
Morocco	Assa-Guelmim	24	12	20	4	0	4	0	0	0	24	0	0	24	0	24
	Awsard	66	66	62	4	5	60	1	66	0	0	0	66	0	0	66
	Fask-Tighmert	154	15	109	45	35	95	24	59	95	0	71	0	83	0	154
	Galtat Zemmour[c]	16	16	0	16	16	0	0	0	0	16	0	0	0	16	0
	Laayoune[c]	83	83	0	83	83	0	0	0	0	83	0	0	30	83	0

Inds: individuals; MERS-CoV: Middle East respiratory syndrome coronavirus.

[a] Number of camels sampled according to variables tested in the modelling of spatial variations and risk factors for estimating the propability of detecting MERS-CoV antibodies and RNA.

[b] A small herd comprised ≤ 50 camels, a medium herd between 51 and 150 camels and a large herd between 151 and 300 camels.

[c] Sampling took place at an abattoir.

same statistical models. All the statistical analyses were performed using the software R [33].

Results

In total 1,500 camels were sampled, between February and March 2015, from 86 herds (Figure 1B–D and Table 1). This included 525 camels in Burkina Faso from 43 herds from four regions (Tinakoff, Gorom, Arbinda, PobéMengao); 632 camels in Ethiopia from 28 herds from five regions (Ayssaita-Dubti; Melkawerer; Akaki-Addis Abeba; Metehara; Yabello) and 343 camels in Morocco from 15 herds from five regions (Assa-Guelmim, Fask-Tighmert, Laayoune, Galtat Zemmour, Awsard).

Collinearity tests

Camel's function and sex were strongly associated with each other (Table 2 and Figure 1B–D) (CrV = 0.86), as were region and lifestyle (CrV = 0.78); herd category and region (CrV = 0.70); region and function (CrV = 0.61); herd category and country (CrV = 0.50); function and lifestyle (CrV = 0.41). The strongest association was between region and type of specimens (i.e. farm or abattoir) with a Cramer's V equal to 1. Region and age were also slightly collinear with a R2 of 0.20.

Modelling spatial variations

At the country scale, seropositivity and virus detection rates varied significantly across regions with p-values < 0.005 for the regional effect (i.e. seropositivity and virus detection rates) (Table 3 and Figures 1 B–D).

In different regions of Burkina Faso, seropositivity rates ranged from 73.2% (95% confidence interval (CI): 48.6–88.8) to 84.6% (95% CI: 77.2–89.9) and virus detection from 0% (95% CI: 0–0) to 12.2% (95% CI: 7–20.4) (Figure 1B). In Ethiopia seropositivity rates ranged from 85.1% (95% CI: 71.8–92.7) to 99.4% (95% CI: 95.4–99.9) and the viral RNA detection rates from 0% (95% CI: 0–0) to 15.7% (95% CI: 8.2–28.0) (Figure 1D). In Morocco, seropositivity rates ranged from 48.3% (95% CI: 18.3–79.5) to 100% (95% CI: 100–100) and viral RNA detection rates from 0% (95% CI: 0–0) to 7.6% (95% CI: 1.9–26.1) (Figure 1C).

Taking the countries globally (irrespective of regional variation), seropositivity and viral RNA detection rates were higher in Ethiopia, as compared with Burkina Faso and Morocco.

Modelling risk factors

In the modelling of variations of seropositivity rates (Figure 2A and Table 3), the retained explanatory variables were herd size category (p-value = 0.061), camel's function (p-value = 0.01) and lifestyle (p-value < 0.005). Higher seropositivity rates were observed (i) in large/medium herds as compared with small herds; (ii) in camels bred for meat or milk as compared with camels bred for transport, and (iii) in nomadic or sedentary herds than in herds with a mix of these lifestyles. Seropositivity rates also increased with age (p-value = 0.032; Figure 3) and were higher in females than in males.

Table 2

Colinearity index among variables explaining MERS-CoV seropositivity and viral RNA detection rates

Colinearity index	Age	Sex	Function[a]	Region	Lifestyle[b]	Type[c]	Herd category[d]	Country
Age	1.00							
Sex	0.03	1.00						
Function[a]	0.04	**0.86**	1.00					
Region	0.20	0.38	**0.61**	1.00				
Lifestyle[b]	0.01	0.12	0.17	**0.78**	1.00			
Type[c]	0.01	0.05	**0.41**	**1.00**	0.25	1.00		
Herd category[d]	0.03	0.22	0.18	**0.70**	0.40	0.19	1.00	
Country	0.13	0.14	0.31	**1.00**	**0.41**	0.25	**0.50**	1.00

Two-by-two comparisons of explanatory variables were used to assess possible confounding influences. Cramer's V test was used for categorical variables and R^2 obtained from linear models for continuous variables. When the statistic is close to 1 for R^2 or larger than 0.4 for CrV test, the two explanatory variables are considered as collinear and cannot be used in the same statistical models. When the statistic is larger than 0.4 for the CrV test the result is in bold.

[a] The function refers to whether the camel was bred for milk, meat or transport.

[b] The lifestyle refers to whether the camel was sendentary, nomadic or had a mix of sedentary and nomadic lifestyles.

[c] The type refers to whether samples were taken, such as a farm or a slaughterhouse.

[d] The herd category refers to the herd size (small with ≤ 50 camels, medium with 51 to 150 camels and large with 151 to 300 camels).

In the modelling of virus RNA detection rate (Figure 2B and Table 3), camel's function had a significant effect (p-value = 0.01) with higher viral RNA detection rates observed in camels bred for milk or for meat as compared with transport. Probability of detecting virus RNA also decreased with increasing age (p-value = 0.06; Figure 3) and was higher in females than in males (according to collinearity index as the variables function and sex strongly associated).

Discussion

Our results support the contention that the MERS-CoV is actively circulating in camel populations in Burkina Faso, Ethiopia, Morocco and likely across all North, West and East Africa. The finding of high levels of seropositivity rates, which is an indication of infection at some point in the animals' life time, was not surprising, and was in keeping with data from previous studies in Ethiopia and in other parts of Africa [19,20,34]. This study, however, presents the first evidence of MERS-CoV activity in Burkina Faso and in Morocco (Figure 1A and previous mapping of MERS serological studies). There are few reports of virus detection in camels in Africa. Here, MERS-CoV RNA was detected at a relatively high rate of up to 15.7% (95% CI: 8.2–28.0) in Ethiopia, followed by Burkina Faso with up to 12.2% (95% CI: 7–20.4) and Morocco up to 7.6% (95% CI: 1.9–26.1).

There is an apparent gradient of virus RNA positivity adjusted for age (Table 3) from west to east which could be explained by a gradient in camel density (Figure 1A), in addition to other drivers such as climate, migratory roads and national and international camel exchanges. Since Ethiopia is a main exporter to the Arabian Peninsula through two main ports in Djibouti and Somalia [35], the virus transmission dynamics in this region is of particular interest.

We observed an increase in seropositivity rate with age which confirms the trend observed in Ethiopia in a previous study [20]. We found a higher virus RNA detection rate in young animals compared with older animals which could be related to a lack of prior immunity as published in previous studies in Saudi Arabia [36]. Young animals were naïve and more susceptible to virus infection (Figure 3) [37].

The role of camel density in shaping the large spatial scale (i.e. national) variation pattern in seropositivity and virus RNA detection rates is supported by the identification, at fine scale (i.e. herd), of a herd size effect on serological prevalence. Higher seropositivity rate was found in large or medium size herds as compared with small herds, suggesting that the transmission of the virus is density dependent. More studies are now necessary to better describe the virus transmission dynamics within herds and between herds, with mechanistic models accounting for a disease transmitted through close contact and the possibility of reinfections [38]. Such a model would allow to determine the minimum size of a camel herd required for the MERS-CoV to persist in that herd without 'fadeouts': i.e. critical community size [39].

Another point highlighted by our study as a risk factor is the function of camels which is also related to sex. Camels raised for milking (which are females) show the highest serological prevalence followed by camels raised for their meat (which are mostly males) and lastly, camels used for transport activities (which are also mostly males), which have the lowest seroprevalence

Table 3

Multivariate modelling used to depict variations in serological and virological status according to individual characteristics (sex and age), spatial localisation (country and regions) and farming practices (camel's function, herd category, and lifestyle) using data from Morocco, Burkina Faso and Ethiopia, February–March 2015

GLOBAL MODEL Multivariate models herd as random effect (1|herd)			
VARIABLES	**AIC**	**Variables**	**P value**
SEROLOGY			
Spatial variations			
Age + country + sex + (1|herd)	1,047.8	NA	NA
Age + region + sex + (1|herd)	1,029.9	Age	0.001
		Region	<0.005
		Sex	0.068
Farming risk factors			
Age + sex + lifestyle[a] + herd category[b] + (1|herd)	960.9	NA	NA
Age + sex + type[c] + lifestyle[a] + herd category[b] + (1|herd)	960.4	NA	NA
Age + function[d] + lifestyle[a] + herd category[b] + (1|herd)	961.4	Age	0.032
		Function[d]	0.016
		Lifestyle[a]	<0.005
		Herd category[b]	0.061
VIRUS DETECTION RATE			
Spatial variations			
Age + country + sex + (1|herd)	640.5	NA	NA
Age + region + sex + (1|herd)	619.7	NA	NA
Age + region + (1|herd)	618.8	Age	0.369
		Region	<0.005
Farming risk factors			
Age + function[d] + lifestyle[a] + herd category[b] + (1|herd)	651.6	NA	NA
Age + function[d] + lifestyle[a] + (1|herd)	647.8	NA	NA
Age + sex + (1|herd)	651.7	NA	NA
Age + function[d] + (1|herd)	646.1	Age	0.067
		Function[d]	0.015

AIC: Akaike information criterion.

AIC Selection and p values. Each model depicts the variation of serological and virological status (response variable: positive/negative results) according to explanatory variables (age, country, region, sex, lifestyle, herd category, camel function). Herd random effects are included in the models (1 |herd). Selection among models including different combinations of these explanatory variables was performed using AIC where a difference of 2 is required for selecting a model which combined variables influencing significantly the response variable.

[a] The lifestyle refers to whether the camel was sendentary, nomadic or both.

[b] Herd category refers to the size of the herd (small with ≤ 50 camels, medium with 51 to 150 camels and large with 151 to 300 camels).

[c] The type refers to whether samples were taken such as a farm or a slaughterhouse.

[d] The function refers to whether the camel was bred for milk, meat or transport.

(Table 3 and model selection). The higher seropositivity rate in females bred for milking could be related to the high viral RNA detection rates in younger animals, e.g. calves [37]. A plausible hypothesis could indeed be that young camels who lack antibodies have a high probability of being infected and in turn expose the mothers to infection or reinfection. The lower seropositivity rate in camels bred for their meat or for transport activities, which are mostly males, could also be linked with the fact that males are often separated from the herd (the two sexes are only mixed during the reproduction activities) and have thus less contacts with other camels (i.e. females and calves).

Surprisingly, there was no observed difference between nomadic and sedentary herds in the seropositivity rate or virus RNA positive rate. Two hypotheses may explain this pattern. Firstly, the sedentary lifestyle is found in animal production systems where animals live at high density in 'commercial' farms. In such situations the virus may be introduced more easily to the herd with animals being bought from other sources and the virus once introduced will amplify to infect most of the susceptible animals, since they are in close contact with each other. The virus appears to have a density dependent transmission pattern. In contrast to this, nomads are long-distance travellers who connect different regions. Consequently they have multiple opportunities to come into contact with other camel populations during their travels, or through indirect contacts with water points and thus increasing the probability of encountering animals shedding MERS-CoV. In support

to these interpretations, the lowest seroprevalence was found for the mixed lifestyle which is associated with medium herd sizes and relatively small range movements.

Our survey was limited to a narrow period in time, February–March 2015, and does not provide insights into seasonal variation in epidemiological dynamics. However, the synchronicity of the study across the different study sites is important because virus shedding may be related to seasonal and breeding cycles across these diverse geographical regions. By keeping this variable within relatively narrow bounds, we are able to meaningfully analyse the other parameters that impact on virus transmission dynamics within dromedary populations. Further studies should follow camel populations through the year to define seasonal variation in virus activity.

The results of our study are coherent with risk factors highlighted by Alraddadi and colleagues for human illness in Saudi Arabia [40]. They show, using a case–control design for exploring environmental exposures among primary case-patients from March to November 2014, that direct exposure to dromedary camels and particularly milking camels was significantly associated to MERS-CoV illness. These results consolidate the risk factors identified in our study on the camel females and milking activities [40]. Our results also give rise to a number of research questions to be followed up in future studies on MERS-CoV transmission dynamics in camel herds. In particular, the role played by young camels and the relationship with the mother need to be investigated more thoroughly.

Longitudinal investigations should also be undertaken in naturally-infected camels in different production systems and different age groups. Such investigations could provide valuable information on virus shedding in excretions (nasal, faecal, milk and urine) and on whether the virus is present in meat. It could also give insights into the dynamics of immunity in camels and reinfection mechanisms. Joint research on risk factors for transmission of MERS-CoV between camels, from camels to humans and from humans to camels should also be encouraged.

Genetic and phenotypic characterisation of MERS-CoV from Burkina Faso, Ethiopia and Morocco is required to understand how MERS-CoV in camels evolves within the continent, particularly with regard to capacity for inter-species transmission to humans.

Our study is one of the few studies that have so far addressed the influence of dromedary lifestyle on MERS-CoV infection as assessed by rate of seropositivity. While the study by Deem et al. (2015) in Kenya did not identify the factors associated with variation of seropositivity among farms [41], our study, which included different countries with a larger geographical range and included a larger number of farms with defined herd size, herd lifestyles and camel functions allowed us to explore associations of these factors with seropositivity. Such data contribute to understanding factors contributing to MERS-CoV infection in camels, which in turn might also have an effect on zoonotic infection. While we carried out our study in different parts of Africa, due to the fact that we have encompassed diverse geographical and ecological variables, our study findings may well be relevant in regions such as Saudi Arabia where zoonotic MERS remains a recurrent threat. Furthermore, it is not clear that transmission of MERS-CoV to humans is absent in Africa. A recent study has reported evidence of humans with MERS-CoV seropositivity in Kenya [22]. Further studies are needed to assess whether or not zoonotic MERS-CoV transmission occurs in Africa and our epidemiological data provide identification of situations of highest risk. Better understanding of the risk factors and virus transmission dynamics of MERS-CoV within camels is important in responding to the global health threat posed by MERS-CoV.

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Conflict of interest

None declared.

Authors' contributions

EM/MP/VC/FR designed the study. GA/MNBB/HB/IEB/OFF/GF/AT did the field work. DKWC/RAPMP/TS/BCYN did the serological and virological analyses. EM/VG did the statistical analyses. EM/MP/VC drafted the manuscript and VG/BF revised it. MP manages the MERS-CoV project.

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