Emergency surveillance for novel influenza A(H7N9) virus in domestic poultry, feral pigeons and other wild birds in Bhutan

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Summary

Following the March 2013 outbreak of novel avian influenza A(H7N9) virus in humans and the subsequent isolation of the virus from chickens, ducks and pigeons in China, concerns were raised that the H7N9 virus would spread beyond China through the poultry value chain linking to a number of bordering countries. For this reason, a rapid emergency surveillance exercise took place in Bhutan between May and July 2013 with the objective of determining whether influenza A(H7N9) virus was silently circulating in domestic poultry and wild birds in Bhutan. A total of 1,716 oropharyngeal, tracheal and cloacal swabs together with faecal droppings were collected from poultry, wild birds and feral pigeons throughout the country; these samples included 150 that had been previously collected for surveillance of influenza A(H5N1) virus. Overall, 733 of the samples were tested. A QIAamp[(R)] Viral RNA Mini kit was used to extract viral RNA from a mix of oropharyngeal, tracheal and cloacal swabs

and faecal droppings. The matrix gene of avian influenza type A virus was detected using a specific real-time reverse-transcription polymerase chain reaction (RT-qPCR) assay, and positive samples were further tested in RT-qPCR for simultaneous detection of the H7 and N9 genes. Among the 733 samples tested, 46 (26 prospective, 20 retrospective) were confirmed positive for influenza A, a prevalence of 6.3% (95% CI 4.6 to 8.3). The influenza A-positive samples were from areas in the south of Bhutan that had experienced previous outbreaks of highly pathogenic influenza A(H5N1). None of the samples tested positive for H7N9 strains, providing evidence that influenza A(H7N9) virus was not present in the sampled population. A risk-based approach for surveillance of influenza A(H7N9) and H5N1 is recommended in Bhutan, based on the epidemiology of the disease in China and other countries in South and Southeast Asia.

Keywords

Bhutan – Emergency surveillance – Novel avian influenza A(H7N9) virus – Poultry bird – Wild bird.

Introduction

On 31 March 2013, three human cases of infection with a novel influenza A(H7N9) virus in Eastern China (Shanghai, Anhui Province) were reported to the World Health Organization (WHO) by the public health authorities of the People's Republic of China, in accordance with International Health Regulations (1). Following that report, H7N9 was the subject of increasing global attention because of the growing number of new cases, accompanied by a relatively high mortality rate, in other provinces in China (2, 3). By 31 May 2013, a total of 132 laboratory-confirmed human cases, of which 39 were fatal, had been reported from eight provinces (1). A further two cases were reported in July. In October the same year the disease re-emerged with a second wave of human cases reported from a number of provinces in China (4, 5, 6). At the same time, H7N9 virus was laboratory-confirmed in poultry. Environmental samples and epidemiological and virological studies have shown that human infection appears to be associated with exposure to live poultry or

contaminated environments, including markets where live poultry are sold (1, 7, 8, 9, 10). Thus, the human H7N9 virus has been identified as the product of reassortment of viruses of avian origin (10). Another important feature of this novel influenza virus is that it shows low pathogenicity in wild birds and domestic poultry, infection resulting only in subclinical or mild disease. This is important, because potentially there could be a silent widespread epizootic in China and its neighbouring countries (2). The emergence and discovery of this novel influenza A(H7N9) virus in humans and poultry is of major public health significance and raises many global public health concerns (1, 2, 3, 11).

As an immediate first step, the Food and Agriculture Organization of the United Nations (FAO) initiated and funded emergency surveillance in China and other high-risk countries in South and Southeast Asia with the explicit aim of determining how widely the virus had spread outside the infected provinces/municipalities in China and how far it had spread in neighbouring at-risk countries (12). The information from these studies was expected to be used to strengthen future medium- to long-term surveillance strategies in the region and to monitor incursion and spread of the virus into uninfected areas (12). Bhutan, with other countries in Southeast (China, Vietnam, Cambodia, Loa, Indonesia, Myanmar) and South (Bangladesh, Nepal) Asia, was considered to be at high-risk for the spread of this novel influenza A(H7N9) virus because of its geographical proximity to China, the presence of bird migration pathways and the possibility that spread might occur from neighbouring countries through trade in poultry and/or poultry products (12).

Possible scenarios for the introduction of this novel avian influenza H7N9 virus into Bhutan are:

- imported human cases
- detection of cases in migratory birds but not in poultry or humans
- detection of cases in migratory birds and poultry but not in humans
- detection of cases in migratory birds, poultry and humans.

It was concluded, therefore, that there was a definite risk of incursion of virus into Bhutan and, for this reason, guidelines for surveillance and contingency plans for each of the possible scenarios of introduction were developed.

In February 2010, Bhutan experienced its first outbreak of the highly pathogenic avian influenza (H5N1) virus in backyard poultry farms in the south-west of the country, close to the Indian state of West Bengal (13, 14). Up to January 2014, at least seven district-level outbreaks were reported in six districts of Bhutan. All the outbreaks were quickly controlled through stamping-out procedures and no human cases of H5N1 were reported, but the outbreaks had a serious economic impact on poultry farmers and on the government (14). The emergence of novel influenza A(H7N9) virus in China heightened public health concern in Bhutan and emergency surveillance was recommended.

The main objective of the emergency surveillance programme was to determine whether H7N9 virus was circulating in domestic poultry and wild birds, including feral pigeons, in Bhutan, and how widely the virus had spread outside the current infection foci in China. The information would be used to strengthen Bhutan's capacity for laboratory and clinical surveillance.

Materials and methods

Between May and July 2013, a total of 1,716 oropharyngeal, tracheal and cloacal samples from domestic poultry in backyards and commercial farms, together with fresh faecal droppings of feral pigeons and other wild birds, were collected from carefully selected high-risk villages in 15 districts of Bhutan (Fig. 1, Table I). A risk-based surveillance approach was adopted by selecting villages/sites that were known to have had outbreaks of highly pathogenic avian influenza (HPAI) (H5N1), and to have a relatively high density of poultry with increased human activity, and/or were habitat areas for migratory birds. The surveillance strategy was designed to provide 95% confidence that infection would be detected at a prevalence of 0.04% in an estimated poultry population of 0.2 million using a test

with 100% sensitivity and specificity. To fulfill these requirements 1,490 samples were needed. To allow for any damage and wastage of samples during shipment to the laboratory or at the time of processing, a total of 1,716 samples were collected by the sampling team. The 82 selected villages/sites had similar poultry populations and an average of 21 samples was collected at each one. Systematic random sampling, based on the approximate estimated poultry population in each village/site, was used until the required numbers of samples were collected. Samples from wild birds were taken randomly from sites close to or in contact with domestic poultry.

All samples were transported to the National Centre for Animal Health at Thimphu, with strict maintenance of the cold chain, and were stored at −80°C. For logistical reasons and because of time constraints, only 583 of the prospectively collected samples were tested in PCR assay. To determine whether the novel virus had already been circulating silently in the poultry and wild bird population prior to the first report of the first outbreak in China (Table I), a further 150 stored samples previously collected from poultry and wild birds between January and May 2013 for HPAI (H5N1) surveillance were tested for avian influenza virus and strain H7N9. Thus, a total of 733 samples (583 prospective, 150 retrospective) were tested on the assumption that they might be influenza positive, based on details of when, where and from which species they were collected.

The RNA was extracted from a mix of oropharyngeal, tracheal and cloacal swabs and faecal droppings using a QIAamp® Viral RNA Mini Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions. A manual centrifugation method was used. Samples were amplified using a Superscript® III Platinum® One-step qRT-PCR kit (Invitrogen, Carlsbad, California, United States) in a Bio-Rad IQ^{TM}5 multiplex real-time PCR system for the detection of type A avian influenza viruses by targeting the matrix gene. Primers and probes were supplied by the Australian Animal Health Laboratory (AAHL) of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Geelong, Australia: forward primer 5′- AGATGAGYCTTCTAACCGAGGTCG -3′,

reverse primer 5′- TGCAAANACATCYTCAAGTCTCTG -3′ and probe 5′-FAM-TTTGTATTCACGCTCACCGTGCCCA-BHQ1-3′. Samples that tested positive for avian influenza virus were used for further H7 subtype identification in a Superscript® III Platinum® One-step qRT-PCR kit and an FLI H7 real-time RT-PCR kit (AAHL, CSIRO): forward primer 5′- AYAGAATACAGATWGACCCAGT -3′, reverse primer 5′- TAGTGCACYGCATGTTTCCA -3′ and probe 5′-FAM- TGGTTTAGCTTCGGGGCATCATG -BHQ1-3′. Since none of the samples tested positive for the H7 strain, no further testing was carried out to identify N9 strains.

Results and discussion

Of the 733 samples tested, 46 (6.3%, 95% CI 4.6 to 8.3) were positive for influenza type A virus: 26 of 583 prospectively collected samples (4.5%, 95% CI 2.9 to 6.5); 20 of 150 retrospectively collected samples (13.3%, 95% CI 8.3 to 20). None of the samples tested positive for the H7 strain, indicating that the novel avian influenza A(H7N9) virus was not circulating in the sampled population in Bhutan in July 2013 (Table I).

Similar surveillance findings have been reported in Southeast (Vietnam, Cambodia, Loa, Myanmar, Indonesia) and South (Bangladesh, Nepal) Asian countries where influenza A has been detected but not the novel H7N9 strains (FAO, unpublished reports). This provides some reassurance that A(H7N9) virus is not circulating in poultry and wild birds beyond China, although imported human cases have been reported in Malaysia, Hong Kong and Chinese Taipei (15, 16, 17). Nevertheless, vigilance and targeted surveillance is recommended, particularly during the winter, as previous outbreaks of A(H5N1) in Bhutan have occurred between December and February (13, 14) and because there were reports of a second wave of A(H7N9) human cases in China after October 2013 (5). The FAO and WHO have also warned of a rising number of humans cases and have called on countries neighbouring China to exercise increased vigilance and strengthen preparedness for A(H7N9) and other avian influenza viruses, such as A(H5N1) (11). Although Bhutan has been carrying

out surveillance for H5N1 virus in domestic poultry, water fowl, and wild and migratory birds following outbreaks in Bhutan and neighbouring countries, there was no testing for influenza A(H7N9) prior to the present study, which was the first emergency surveillance study on novel influenza A(H7N9) virus in Bhutan. With the establishment and strengthening of laboratory diagnostic facilities in Bhutan, it is expected that surveillance and preparedness for avian influenza incursions will be enhanced.

The influenza A-positive samples in this study, both prospectively and retrospectively collected, were from the four districts in the south of Bhutan (Samtse, Chukha, Sarpang, Dagana) that had reported outbreaks of influenza A(H5N1) between February 2010 and January 2013 (Fig. 1). This could be an indication of the establishment and adaptation of the virus to the local environmental conditions in these high-risk areas. Chukha district (Fig. 1, Table I) reported the highest prevalence of influenza A and also reported outbreaks of HPAI (H5N1) for the four consecutive years 2010 to 2013 (12, 13). Influenza A viruses were detected in all bird species tested (poultry, feral pigeons, wild birds). Risk-based surveillance is recommended in this area of Bhutan to detect circulating influenza A(H7N9) virus and other influenza strains in poultry and wild birds and, ultimately, to prevent infection in humans.

Conclusions

Tests on 150 samples from poultry and wild birds collected between January and May 2013 and 583 samples collected between May and July 2013 were all negative for avian influenza A(H7N9). If avian influenza A(H7N9) infection was present in Bhutan at the time of sampling it is 95% certain that the prevalence of infection was below 0.04%.

Poultry in areas of Bhutan that had previously recorded outbreaks of HPAI (H5N1) tested positive for influenza A virus. In acknowledging that incursion of virus into the country could occur at any time, a risk-based approach to serological and virological surveillance for A(H7N9) is recommended in Bhutan. The disease situation in China

and other countries in South and Southeast Asia should be monitored on an ongoing basis and testing for influenza A(H5N1) should be carried out, particularly during winter months.

Contingency plans for responding to any possible incursion of virus into the country should be prepared.

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Table I
Overall prevalence of influenza A virus in poultry and wild bird species in Bhutan

District	Number of samples tested			Number of influenza A positives			% Prevalence (95% CI)[c]	
	Wild birds[a]	Poultry[b]	Total	Wild birds	Poultry	Total		
Prospectively collected samples:								
Chukha	93	75	168	11	5	16	9.5	(5.9–15)
Dagana	21	14	35	0	1	1	2.8	(0.5–15)
Samtse	36	43	79	2	2	4	5.1	(1.9–12.3)
Sarpang	45	32	77	5	0	5	6.5	(2.8–14.3)
Haa	6	16	22					
Paro	15	15	30					
Punakha	2	3	5					
Samdrup Jongkhar	23	6	29					
Bumthang	12	5	17					
Thimphu	38	10	48					
Trashigang	4	7	11					
Trashiyangtse	2	6	8					
Trongsa	9	9	18					
Tsirang	11	16	27					
Wangdue	2	7	9					
Total	319	264	583	18	8	26	4.5	(3.1–6.5)
Retrospective samples:								
Chukha	27	28	55	6	4	10	18.2	(10.2–30.3)
Samtse	15	17	32	0	5	5	15.6	(6.9–31.7)
Sarpang	15	15	30	1	4	5	16.6	(7.3–33.6)
Dagana	4	2	6					
Punakha	3	1	4					
Samdrup Jongkhar	11	1	12					
Bumthang	2	0	2					
Thimphu	0	2	2					
Trashiyangtse	1	0	1					
Tsirang	4	1	5					
Wangdue	1	0	1					
Total	83	67	150	7	13	20	13.3	(8.8–19.7)

a) Includes crows, feral pigeons and other wild birds

b) Includes chickens, ducks and geese

c) Influenza A-positive samples per 100 samples tested

Fig. 1
Influenza A(H7N9) emergency surveillance sampling sites, A(H5N1) outbreak areas (February 2010 to January 2013) and areas of avian influenza A virus-positive poultry and wild birds in Bhutan