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Avian Influenza H5N1 Surveillance in Geese of Qinghai Province, China (2012)

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Abstract

The aim of study was to detect H5N1 virus in wild geese in Qinghai Province in 2012. The work was provided according to WHO and OIE guidelines. In 2012, we collected 532 samples from wild geese of two species: Bar-headed Goose (Anser indicus) and Graylag Goose (Anser anser). We analyzed samples by chicken embryo inoculation and PCR. No avian influenza viruses were isolated. History of HPAI H5N1 shows obvious importance of Central Asian region in its spreading. The outbreaks of the H5N1 Highly Pathogenic Avian Influenza (HPAI H5N1) were reported in wild birds at the Qinghai Lake since 2005. This area seems to be key point for H5N1 avian influenza surveillance in wild birds. We did not find viruses although H5N1 cases in poultry were reported from 5 provinces of China in 2012. Annual surveillance is required for early AIV detection in this region.

Keywords

Avian Influenza; H5N1; Geese; Qinghai Province; China

1. Introduction

Highly pathogenic H5N1 influenza viruses (HPAI H5N1) in spite of intensive control measures are known to be serious threat to humans. Since 2003, the number of human cases of avian influenza A (H5N1) reported from 15 countries worldwide is 644, of which 381 were fatal (as of 8 November 2013) [1]. The latest human avian in-

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fluenza case in China was reported by Guizhou Province CDC on 10th February 2013. A 31 years old male from Guiyang district was confirmed positive for A/H5N1 influenza [1].

History of HPAI H5N1 shows obvious importance of Central Asian region in its spreading [2]-[8]. Virus has been reported in Qinghai-Tibetan Plateau since 2005 and caused numerous outbreaks in different wild birds and poultry [2] [4]-[6].

In spring 2005 at Qinghai Lake, an unprecedented outbreak of HPAI H5N1 caused the death of more than 6000 migratory birds including over 3000 bar-headed geese (Anser indicus) and in following years has re-emerged in wild birds along the Central Asia flyway several times [2] [9].

No case of human avian flu has been reported in northwest China's Qinghai Province where an outbreak was found among numerous migratory birds [1]. However, this province seems to be key point for H5N1 avian influenza surveillance in wild birds. For these reasons, we continue annual Surveillance program in Qinghai Lake area.

2. Methods

All the samples were collected, transported, stored and tested according to WHO and OIE Manuals [10] [11]. The swabs and feces were collected in 2-ml tubes with transport media containing phosphate-buffered saline (140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2), sterile glycerine in 1:1 volume proportion and antibiotics: penicillin, streptomycin, gentamicin, and nystatin in concentrations 2×10^6 m/L, 0.20 g/L, 0.25 g, and 0.5×10^6 m/L, respectively [11] and immediately frozen and stored at −196C (in liquid nitrogen) until virus isolation was attempted. Swabs and feces samples were tested for influenza viruses by inoculation into the allantoic cavity of 10-day-old embryonating specific-pathogen-free chicken eggs according to standard procedures and by real-time RT-PCR [11]. Each sample underwent at least three passages in chicken eggs, and influenza isolates were identified by both HA assay and real-time RT-PCR [11].

HA assay was performed with mcl phosphate-buffered saline (pH 7.2). We added 50 mcl of 0.5% chicken erythrocytes suspension in each well of the plate to 50 mcl of allantoic fluid 2-fold dilutions. Then stored at +4C temperature for 60 min and checked the results according to WHO Manual [11]. We also conducted negative and positive controls of 0.5% chicken erythrocytes suspension, and negative controls of allantoic fluid of pathogen specific free embryos with 0.5% chicken erythrocytes suspension.

Viral RNAs were isolated from feces, swabs and allantoic fluid by trisol-chloroform methods (http://molbiol.edu.ru/protocol/15_10.html). Reverse transcription (RT) reactions were made with the RevertAidTM M-MuLV Reverse Transcriptase (Fermentas, Lithuania) and the PrimeScript™ RT Reagent Kit (TaKaRa, China) as specified by the manufacturer. A random Hexamer Primer was used for RT.

The cDNAs of the AIV were detected by Real-Time PCR with Taq DNA polymerase (Medigen Laboratory, Russia) and a Premix Ex Taq™ (TaKaRa, Japan). Taqman probes (5′-TCGAAACGTACGTTCTCTCTATC-3′, 5′-FAM-TCAGGCCCCCTCAAAGCCGA-Q-3′, 5′-TGTCTTCAGCCATTCCATGAG-3′), specific for the viral M gen, were used.

3. Results

In June of 2012, we collected 100 cloacal swab samples from Bar-Headed Geese at Qinghai Lake. In September-October 2012 we collected 322 samples from Graylag geese (Anser Anser) at Gengga Lake 110 samples from Bar-headed geese (Anser Indicus) at Qinghai Lake. Totally we collected 532 samples from geese (**Table 1**).

Table 1. Geese samples collected in Qinghai Province, China in 2012.

Bird species	Number of samples	Type of sample	Date of sampling	Sampling Area
Bar-headed goose (Anser indicus)	100	Cloacal and tracheal swabs	15.06.2012	Qinghai Lake
	110		12.10.2012	
Graylag goose (*Anser anser*)	126	Feces	13.09.2012	Gengga Lake
	158	Feces	19.09.2012	
	38	Feces	25.09.2012	

Swabs and feces samples were tested for influenza viruses by real-time RT-PCR first. No positive specific results were found. Then samples were tested by inoculation into the allantoic cavity of 10-day-old embryonating specific-pathogen-free chicken eggs. Each sample underwent at least three passages in chicken eggs, and after each passage influenza isolates were identified by both HA assay and real-time RT-PCR [11]. No positive allantoic fluids were found to be positive in HA assay and real-time RT-PCR.

In result of study we did not isolate any influenza viruses from collected material by inoculation of chicken embryos. Also we did not find positive samples using PCR method. According to WHO and OIE guidelines we can conclude that our samples collected from geese were negative for presence of avian influenza viruses.

4. Discussion

As Qinghai and Gengga Lakes serves as one of the key stopovers on the flyway between South East Asia and Europe, early AIV detection in that areas can play an important role in terms of outbreak prediction, early warning and isolating new AIV strains when they start spreading from Asia to Russia and Europe [2] [5] [6] [12]. We did not find the H5N1 viruses in 2012, however, our data correlate with OIE source information. No one H5N1 case was registered in Qinghai Province in 2012. Some H5N1 cases in poultry were reported from 5 provinces of China (Gansu, Liaoning, Ningxia, Yunnan, Guangdong) when about 14,000 domestic birds died and more than 1,300,000 were destroyed [13].

There were reported several H5N1 outbreaks in wild birds at Qinghai Lake before since 2005 [2]-[6]. H5N1 was isolated mainly from the following bird spices: bar-headed geese (Anser indicus), great cormorants (*Phalacrocorax carbo*), great black-headed gulls (*Ichthyaetus ichthyaetus*) and great-crested grebes (*Podiceps cristatus*) [2] [5]. An important feature of Qinghai Province is that there is a little domestic poultry production in the country, therefore earlier AIV detection in wild birds would not likely be from the domestic poultry [2] [5] [6] [13].

Last survey on current waterbirds community at Gengga Lake of Qinghai Province showed the importance that one for the surveillance of avian influenza and the wetland conservation and management at Gengga Lake. A total of 27 416 individuals of 55 waterbird species, belonging to 12 families and 6 orders, were recorded in the survey. Geese and ducks were the most abundant species, accounted for 69.0% of all waterbirds counted. Thousands of waterbirds often aggregated at resting and feeding areas in October, and many livestock often mixed with the wild fowls, increasing opportunity for infection of avian influenza among intra-and inter-species [12]. However, this study was aimed to detect AIV specifically in geese first, planning to concentrate on wild ducks during second stage. It was necessary to test the hypothesis of key HPAI H5N1 reservoir in Qinghai Province.

Earlier we collected 1600 samples in non-epizootic period from goose feces in 2010 in Qinghai Province that were tested by Real-Time PCR. Out of samples, no AIV were detected [14]. Another studies showed high rate of H5N1 virus during outbreaks when high geese mortality was recorded [2] [5] [6].

5. Conclusion

Obviously, further inquiry of AIV in wild goose populations is required. Studies of wild geese and AIV ecology will allow us to obtain more information about pathogen-host relationships and to make arrangements for the maintenance of wild goose populations that is why the AIV surveillance at Qinghai Province is very important [14]. Diversification of waterbird life status showed importance for AIV sampling in all seasons [15]. Thus, we continue Annual Surveillance of H5N1 because it is necessary for early detection of the virus, investigation of its biological characteristics and for the H5N1 outbreaks' predicting.

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