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Results of the influenza virus surveillance in wild birds in Western part of Mongolia

Marchenko V Yu¹, Alekseev A Yu¹, Tserennorov D², Yurlov AK³, Susloparov IM¹,
Sharshov KA¹, Ilyinykh FA¹, Zolotykh SI¹, Abmed D⁴, Otgonbaatar D², Shestopalov AM¹

¹Federal State Research Institution, State Research Center of Virology and Biotechnology Vector, Koltsovo, Novosibirsk Region, Russia

²National Centre for Diseases with Natural Foci, Ulaanbaatar, Mongolia

³Institute of Systematic and Ecology of Animals, Siberian Branch, Russian Academy of Science, Novosibirsk, Russia

⁴National Centre for Infectious Diseases, Ulaanbaatar, Mongolia

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ABSTRACT

Objective: To present results of virological study of wild birds inhabiting Western Mongolia. **Methods:** Over a period of 2003–2008, we isolated 13 influenza A viruses: H1N1, H3N6, H13N8 and H4N6 subtypes. We did not isolate any H5N1 subtype, that still cause epizooty in wild birds and poultry. **Results:** We revealed taxonomic and ecological heterogeneity of the birds involved in maintenance of circulation of influenza viruses in the given territory. Influenza viruses were isolated from birds of 6 orders; among them there are species preferring water and semi-aquatic biotopes, one species preferring dry plain region, and also one species which can inhabit both dry and water biotopes. **Conclusions:** Representatives of all main orders of Western Mongolia avifauna are involved in support of influenza A virus circulation, highly pathogenic H5N1 influenza viruses were registered in Mongolia thus it's necessary to continue permanent influenza virus surveillance in wild birds' populations.

1. Introduction

Scientists have always paid careful attention to viruses that circulate through the offices of migratory birds since these viruses can be carried over to long distances (including transcontinental), can cause epidemical outbreaks, and can form active feral herd viral infections. Currently there has been detected the role of migratory birds in circulating of viral infections virulent for humans. Influenza viruses are of the utmost interest since they cause epidemics and pandemics leading to mass human mortality and to economic penalties.

Influenza virus surveillance, primarily in wild birds, is extremely relevant because since 1997 endemic focus of H5N1 influenza virus subtype has been forming in Southeastern Asia and in China. H5N1 subtype has led to mass epizooty among poultry and wild birds in this region and caused death of some hundreds of people. It should be emphasized that high pathogenicity for natural hosts-waterfowl and semi-aquatic wild birds is a unique property

of H5N1 viruses that has been circulating in 2002 through 2009.

Influenza A viruses (Orthomyxoviridae, Orthomyxovirus) are isolated from different species of birds and mammals, including humans [1]. Currently circulation of Influenza A viruses of all known subtypes of hemagglutinin and neuraminidase have been registered only in birds [1,2]. Wild birds migrations make viruses of different subtypes spread extensively[3]. To optimize the control over infectious agents spreading, it is important to study circulation of influenza virus in wild birds inhabiting regions on migratory birds' flyways. Western part of Mongolia (Basin of Great Lakes, the habitat and the place of nesting of wide range of birds' species) is one of the regions which station itself on the flyway between Eastern Asian countries and Russia.

The Basin of Great Lakes is a broad intermountain area edged by Mongolian and Gobi Altai in the South in the West (in the South the basin reaches the desert cavity Shargay Gobi), by the shoots of the Khangai in the East, by range Tannu-Ula in the North and occupies 100 000 square kilometers in area. The basin is situated on the boarder of Tuva Republic and Mongolia. Through the northern part of the basin goes small range Khan-Khukhiyn-Nuruu separating Uvs-Nuur Lake and the rest part of the Basin.

At entrance of the basin the river valleys get broader and form broad deltas. In the western part of the basin lies the

*Corresponding author: Marchenko V Yu, Federal State Research Institution, State Research Center of Virology and Biotechnology Vector, Koltsovo, Novosibirsk Region, Russia

Tel: +79232454405

Fax: +7(383)3367540

E-mail: Vasili86@ngs.ru

salt lake Uvs–Nur, the biggest lake in Mongolia. Numerous lakes fill the lowlands of the basin. The main landform in the Basin of Great Lakes is rugged mountains, sloping plains, lake plains, and sandy lodgments of different kinds. The basin is a unique place since it includes almost all natural zones of the Earth in the limited area. On the bed of the basin lie sandy and clay deserts, on foothill plains – dry steppes. On the mountain slopes lie tallgrass steppes that change over to forest steppe. Higher in the mountains grow mixed broad-leaved and cedar forests. And at the tops of the mountains locates tundra and golets. Thus, the most northern deserts in the world are situated in the Basin of Great Lakes and the most southern tundra are in its mountain surround. Old Central–Asian flyway of Western and Central Siberian waterfowl goes through the Basin of Great Lakes. During some hundreds of years generations of swans, geese and ducks fly through the Basin of Great Lakes to the Yellow Sea seashore and then to the places of wintering in Southeastern Asia.

Since endemic nidus of H5 influenza virus subtype started forming in Southeastern Asia and China, mortality of birds has repeatedly been registered and highly pathogenic H5N1 influenza viruses have been isolated in 2006 and 2009 in the Uvs–Nur basin, that is a part of The Basin of Great Lakes.

The present paper is concerned with results of virological study of wild birds inhabiting Western Mongolia. The research was conducted in 2003 through 2008 for the purpose of studying of influenza virus circulation in the given territory.

2. Materials and methods

Field data was collected from wild birds that were shot off or trapped in the period from the middle of August to the middle of September during 2003–2008 in the territory of western part of Mongolia (Bayan–Ulgia region, Hovd region and Ubsunur region). Personnel of Plague Control Stations of above mentioned areas took part in organization of field data collecting.

Field data were represented as cloacal swabs, and pieces of intestinal tract. Sample collection and storage were provided by using Dewar flask with liquid nitrogen in accordance with WHO recommendations [4].

Influenza A virus isolation was carried out by inoculation of cloacal swabs into allantoic sac of chicken embryo in accordance with WHO recommendations [4].

Serological identification of isolates was carried out by serological and microbiological methods with usage of diagnostic serum set to reference influenza A viruses in accordance with WHO recommendations [4].

Viral RNA was isolated from allantoic liquid by means of Total RNA Isolation System (Promega Corporation, USA). For reverse transcription we used general primer Uni12 AMV–reverse Transcriptase (Fermentas, Lithuania). For PCR we used primers specific to each gene[5]. Amplicons were obtained by dint of QIAquick gel extraction kit (Qiagen, USA). They were sequenced with automatic sequenator (Applied Biosystems 3130 xl Genetic Analyzer). Primary analysis was conducted by usage of Vector NTI Advance 10 (Invitrogen, USA).

3. Results

Avifauna of studied region (The Basin of Great Lakes) numbers 210 species referred to 17 orders. About 80% of species in this region belong to 4 orders–*Anseriformes*, *Charadriiformes*, *Passeriformes*, *Falconiformes*.

As a result of joint expeditions the field data were collected from 443 wild birds. Examined birds belonged to 57 species and to 10 orders (Table 1).

33.2% (147) of examined birds belong to *Anseriformes* (12 species); 13.3% (59 bird units) – to *Charadriiformes* (13 species); 21.2% (94 bird units) –to *Passeriformes* (18 species); 1.6 % (7 bird units) – *Ciconiiformes* (3 species); 6.5% (29 bird units) – *Gruiformes* (2 species); 6.5% (29 bird units) – *Podicipediformes* (2 species); 14.5% (64 bird units) – *Pelecaniformes* (1 species); 1.6% (7 bird units) – *Falconiformes* (3 species); 1.4% (6 bird units) – *Galliformes* (2 species); 0.2% (1 bird unit) – *Columbiformes* (1 species).

The most spread species are: great cormorant (*Phalacrocorax carbo*) (14.4%), Gadwall (*Anas strepera*) (13.1%), Herring gull (*Larus argentatus*) (6.8%), Great-crested grebe (*Podiceps cristatus*) (6.3%), bald-coot (*Fulica atra*) (5.9%), Roody shelduck (*Tadorna ferruginea*) (5.9%), Tree sparrow (*Passer montanus*) (5.2%), Pochard (*Aythya ferina*); (4.2%), White wagtail (*Motacilla alba*) (4.3%).

Influenza viruses were isolated from 11 species *Anseriformes* [Tufted duck (*Aythya fuligula*)], Velvet scoter (*Melanitta fusca*), Common merganser (*Mergus merganser*), Mallard duck (*Anas platyrhynchos*), Roody shelduck (*Tadorna ferruginea*); *Charadriiformes* [Herring gull (*Larus argenatus*)]; *Podicipediformes* (*Great-crested grebe* (*Podiceps cristatus*)); *Pelecaniformes* [Great cormorant (*Phalacrocorax carbo*)]; *Passeriformes* [Magpie(*Pica pica*)] *Isabelline wheater* [(*Oenanthe isabellina*)] and *Falconiformes* [Marsh harrier (*Circus aeruginosus*)] (Table 2).

4. Discussion

The biggest part of influenza A viruses were isolated from species ecologically associated with aquatic and semi-aquatic areas and belong to the following orders: *Anseriformes* (tufted duck, black scoter, goosander, mallard, roody shelduck) *Pelecaniformes* (*Cormorant*). *Isabelline chat* (*Oenanthe isabellina*) inhabit dry plaint areas, magpie (*Pica pica*) inhabit scrub irrespectively of water reserves presence[6].

Influenza A viruses were isolated from 90 species belonged to 12 bird's orders. However the role of each species in influenza virus circulating has not been yet defined. Papers concerning examination of wild birds showed that the main influenza virus reservoir among birds is birds ecologically connected with water – waterfowls of coastline [1,7]. However among this ecological group of birds, heterogeneity of influenza virus circulating was detected. Although most viruses are isolated from waterfowls, H13 is mostly isolated from coastline birds and gulls [8–10].

Thus we isolated influenza A virus from Herrin gull (*Larus argenatus*), trapped at The Uvs– Nur Lake. The isolate was subtyped as H13N8 influenza virus.

The analysis of hemagglutinin gene fragment of the strain (A/herring gull/Mongolia/454/08) showed that this strain is philigenetically similar to strains with various type of neuraminidase, isolated in noth–west part of

Table 1

Species and number of studied birds.

Order	Species of studied birds	Number of studied samples	
<i>Anseriformes</i>	Bar-headed goose (<i>Anser indicus</i>)	4	
	Roody shelduck (<i>Tadorna ferruginea</i>)	26	
	Stiff-tailed duck (<i>Oxyura leucocephala</i>)	5	
	Tufted duck (<i>Aythya fuligula</i>)	7	
	Bullhead (<i>Bucephala clangula</i>)	5	
	Pochard (<i>Aythya ferina</i>)	20	
	Gray goose (<i>Anser anser</i>)	2	
	Mallard (<i>Anas platyrhynchos</i>)	5	
	European teal (<i>Anas crecca</i>)	1	
	Gadwall (<i>Anas strepera</i>)	58	
	Velvet scoter (<i>Melanitta fusca</i>)	11	
	Common merganser (<i>Mergus merganser</i>)	3	
	<i>Charadriiformes</i>	Sea-swallow (<i>Sterna hirundo</i>)	2
		Plover (<i>Charadrius pluvialis</i>)	1
Phalarope (<i>Steganopus tricolor</i>)		2	
Herrin gull (<i>Larus argentatus</i>)		30	
Black-headed gull (<i>Larus ridi bundus</i>)		5	
Sandpiper (<i>Calidris temminckii</i>)		4	
Lapwing (<i>Vanellus vanellus</i>)		2	
Redshank (<i>Tringa totanus</i>)		1	
Black-bellied sandpiper (<i>Calidris alpina</i>)		2	
Upland plover (<i>Bartramia longicauda</i>)		3	
Common sandpiper (<i>Actitis hypoleucos</i>)		3	
Curlew sandpiper (<i>Calidris testacea</i>)		3	
Turnstone (<i>Arenaria interpres</i>)		1	
<i>Passeriformes</i>		Carrion crow (<i>Corvus corone</i>)	7
	Magpie (<i>Pica pica</i>)	6	
	Common wheatear (<i>Oenanthe oenanthe</i>)	10	
	White wagtail (<i>Motacilla alba</i>)	19	
	Paddy-field warbler (<i>Acrocephalus agricola</i>)	8	
	Tree sparrow (<i>Passer montanus</i>)	23	
	Hodgson's bushchat (<i>Saxicola insignis</i>)	1	
	Killigrew (<i>Pyrrhocorax pyrrhocorax</i>)	1	
	Daw (<i>Corvus monedula</i>)	1	
	Isabelline wheatear (<i>Oenanthe isabellina</i>)	3	
	Red-backed shrike (<i>Lanius collurio</i>)	1	
	Horned lark (<i>Eremophila alpestris</i>)	1	
	Starling (<i>Sturnus vulgaris</i>)	2	
	Mountain chat (<i>Oenanthe monticola</i>)	2	
	Desert wheatear (<i>Oenanthe deserti</i>)	5	
	Bluethroat (<i>Luscinia svecica</i>)	2	
	Brambling (<i>Bucanete mongolicus</i>)	1	
	Yellow-headed wagtail (<i>Motacilla citreola</i>)	1	
	<i>Ciconiiformes</i>	Great white heron (<i>Ardea alba</i>)	4
Common heron (<i>Ardea cinerea</i>)		2	
Spoonbill (<i>Platalea leucorodia</i>)		1	
<i>Gruiformes</i>	Corncrake (<i>Crex crex</i>)	3	
	Bald-coot (<i>Fulica atra</i>)	26	
<i>Podicipediformes</i>	Great-crested grebe (<i>Podiceps cristatus</i>)	28	
	Little grebe (<i>Tachybaptus ruficollis</i>)	1	
<i>Pelecaniformes</i>	Great cormorant (<i>Phalacrocorax carbo</i>)	64	
<i>Falconiformes</i>	Black kite (<i>Milvus migrans</i>)	4	
	Marsh harrier (<i>Circus aeruginosus</i>)	2	
	Rough-legged hawk (<i>Buteo lagopus</i>)	1	
<i>Galliformes</i>	Quail (<i>Coturnix coturnix</i>)	1	
	White grouse (<i>Lagopus lagopus</i>)	5	
<i>Columbiformes</i>	Eastern turtledove (<i>Streptopelia orientalis</i>)	1	

Table 2

Results of typing of influenza viruses isolated from birds trapped or shot in Western Mongolia in 2003–2008.

Species	Strain	Subtype	Year of isolation
Magpie (<i>Pica pica</i>)	A/magpie/Mongolia/17/03	H4N6	
Isabelline wheatear (<i>Oenanthe isabellina</i>)	A/isabelline wheatear/Mongolia/19/03	H4N6	
Great-crested grebe (<i>Podiceps cristatus</i>)	A/grebe/Mongolia/24/03	H4N6	2003
	A/cormorant/Mongolia/5/03	H4N6	
Great cormorant (<i>Phalacrocorax carbo</i>)	A/cormorant/Mongolia/2/03	H4N6	
	A/cormorant/Mongolia/22/04	H3N6	
Tufted duck (<i>Aythya fuligula</i>)	A/tufted duck/Mongolia/22/04	H3N6	
Velvet scoter (<i>Melanitta fusca</i>)	A/velvet scoter/Mongolia/19/04	H3N6	2004
Common merganser (<i>Mergus merganser</i>)	A/common merganser/Mongolia/29/04	H3N6	
Marsh harrier (<i>Circus aeruginosus</i>)	A/marsh harrier/Mongolia/30/04	H3N6	
Mallard (<i>Anas platyrhynchos</i>)	A/Mallard/Mongolia/2307/2006	H1N1	2006
Roody shelduck (<i>Tadorna ferruginea</i>)	A/roody shelduck/Mongolia/632/2007	H1N1	2007
Herring gull (<i>Larus argentatus</i>)	A/herring gull/Mongolia/454/08	H13N8	2008

Europe and in European part of Russia in 1998–2005: A/gull/Astrakhan/1818/1998 (H13N6), A/Larus minutus/Astrakhan/3357/2002 (H13N6), A/black-headed gull/Netherlands/1/00 (H13N8), A/black-headed gull/Sweden/1/99 (H13N6), A/yellow-legged gull/Ukraine/912306/2005 (H13). This fact points out at a possibility of migration connections of wild birds that are natural hosts in the extensive territory of Eurasia and at unanimity of influenza virus genofond in given territory. Notice that it is known only 2 characterized H13N8 strains isolated in Netherland (A/black-headed gull/Netherlands/1/00) and in Norway (A/black-headed gull/Norway/10_1459/2006); the information about these strains is available in International Database (GenBank).

Among waterfowls *Anseriformes* have a leading role in quantity and diversity of viruses isolated from them [9], this statement is approved by results of this paper. However sensitivity to influenza viruses of different species belonged to one order can vary. Experimental infection of chickens and quails belonged to the *Galliformes* order showed that the latter ones are more sensitive to influenza A virus, than chickens [11].

The results of our research indicated that variety of species belonged to several orders support influenza virus circulation in Western part of Mongolia. They are birds of aquatic and semi-aquatic biotopes, species that prefer dry plain territory and that can inhabit both dry and water territories.

Landscape of Western part of Mongolia consists of different kinds of habitats, which change each other in latitudinal and altimetric directions. In Mongolia water ecosystems situated inside desert-steppe areas.

For Birds' spreading in this region wide interpenetration of fauns common for different zones [12].

Apparently, taxonomical and ecological heterogeneity of bird species, involved in influenza virus circulation in the territory of Western Mongolia is explained by low number of appropriate places for waterfowl nesting. This can be a favorable condition for restoration of genes of different influenza virus strains.

Thus, representatives of all main orders of Western Mongolia avifauna are involved in support of influenza A virus circulation. Circulation of H3N6, H4N6, H1N1, H3N8 subtypes was proved. In our researches highly pathogenic H5N1 virus was not detected. However taking into consideration that during the period of 2003 through 2008 highly pathogenic H5N1 influenza viruses were registered in Mongolia it is necessary to continue permanent influenza virus surveillance in wild birds' populations.

Conflict of interest statement

We declare that we have no conflict of interest.

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