

Sparse evidence for equine or avian influenza virus infections among Mongolian adults with animal exposures

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In recent years, Mongolia has experienced recurrent epizootics of equine influenza virus (EIV) among its 2.1 million horses and multiple incursions of highly pathogenic avian influenza (HPAI) virus via migrating birds. No human EIV or HPAI infections have been reported. In 2009, 439 adults in Mongolia were enrolled in a population-based study of zoonotic influenza transmission. Enrollment sera were examined for serological evidence of infection

with nine avian, three human, and one equine influenza virus strains. Seroreactivity was sparse among participants suggesting little human risk of zoonotic influenza infection.

Keywords Agriculture, communicable diseases, emerging, influenza A virus, occupational exposure, seroepidemiologic studies, zoonoses.

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With vast and diverse terrains, harsh climates, and large populations of wild and domestic animals, Mongolia is home to numerous zoonotic disease problems. Influenza A viruses have caused considerable morbidity and deaths to horses and migrating birds. Approximately every 10 years, Mongolia experiences large epizootics of equine influenza virus (EIV) among its 2.1 million horses.¹ Anecdotal reports suggest that Mongolian children become sick when horses are sick with EIV. Since first detections were noted in 2005, Mongolia's large migrating bird populations harbor both highly pathogenic and low pathogenic avian influenza viruses (AIV).^{2–5} As both EIV⁶ and AIV are known to infect man, we sought to study Mongolians for evidence of EIV and AIV infection.

Methods

Four institutional review boards approved the study. Eligible participants (≥ 18 years old and self-reporting no immunocompromising conditions) were recruited from seven soums (counties) within three aimags (provinces; Figure 1). In general, participants worked in livestock, agriculture, and mining industries. Consenting participants were interviewed

at their home by study staff who completed enrollment forms and collected blood samples via venipuncture. Demographic information and medical history, including prior receipt of influenza vaccines and recent respiratory illness history, were assessed. Participants reported community, household, and occupational animal exposures. Reports of disease outbreaks in the participants' flocks/herds were also recorded. Data from the enrollment questionnaire were used to dichotomously classify domestic and wild animal exposures based upon a cut point of ≥ 5 cumulative hour/week during one's lifetime. Non-animal-exposed controls with no self-reported household and occupational animal exposure were recruited from the capital city of Ulaanbaatar.

Laboratory methods

Whole blood specimens were transported using cold chain within 24 hours after collection to local field laboratories in Khovd and Dornogovi provinces and to the National Influenza Center of the National Center of Communicable Diseases, in Ulaanbaatar. Upon arrival, blood specimens were accessioned, and serum was separated, aliquoted, and frozen at -80°C . Frozen sera were transported on dry ice to the University of Florida for testing.



Figure 1. Country map of Mongolia showing aimags (provinces) where animal-exposed participants were enrolled (1-Khovd, 2-Tuv, and 3-Dornogovi). Most non-exposed participants were enrolled in the capital city (4-Ulaanbaatar).

Influenza virus strains were selected based upon the hemagglutinin (HA) type for their best geographic and temporal proximity to the study population (Table 1). A microneutralization (MN) assay adapted from previous reports by Rowe *et al.*^{7–11} was used to detect antibodies against a panel of avian and avian-like influenza A viruses, as well as a Mongolian H3N8 EIV.

Due to a low prevalence of elevated antibodies against the various avian and equine influenza viruses, rapidly waning titers,¹² and the inability to determine when an infection

might have occurred, a low threshold of antibody titer ($\geq 1:10$) was chosen as evidence of previous infection with a strain of EIV or AIV. Additionally, cross-reactions from previous infection with human influenza viruses might confound the serology; therefore, potential confounding was controlled by also testing sera for antibodies against three human influenza viruses, using the hemagglutination inhibition (HI) assay as previously described.^{13,14} A HI titer $\geq 1:40$ was used as a cut point in including elevated antibody against human virus for multivariate modeling. MN and HI assay methods are reported as supplemental information.

Table 1. Viruses used in serological studies. Unless otherwise indicated, serologic study was performed using the microneutralization assay

Avian viruses	Human viruses
A/Migratory duck/Hong Kong MPS180/2003(H4N6)	A/Brisbane/59/2007(H1N1)*
A/Cygnus/Mongolia/3/2009(H5N1)	A/Mexico/4108/2009(H1N1)*
A/Nopi/Minnesota/2007/462960-2(H5N2)	A/Brisbane/10/2007(H3N2)*
A/Teal/Hong Kong/w312/97(H6N1)	
A/Water fowl/Hong Kong/Mpb127/2005(H7N7)	Equine virus
A/Migratory duck/Hong Kong/MP2553/2004(H8N4)	A/Equine/Mongolia/01/2008(H3N8)
A/Hong Kong/1073/1999(H9N2) [†]	
A/Migratory duck/Hong Kong/MPD268/2007(H10N4)	

*Virus studied with hemagglutination inhibition assay.

[†]Virus of avian origin but isolated from a human.

Statistical methods

Analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, NC, USA). Comparisons of participant demographics between the exposure groups were made using binary logistic regression. An exact conditional method was used for sparse data.

Results

Between January and June, 2009, 439 participants were enrolled: 358 (81.5%) reported household and/or occupational exposure to animals, and 81 (18.5%) were non-animal-exposed subjects. The cohort's median age was 39, and 52.2% were male (Table 2). Seventeen participants (4.0%) reported having previously received a seasonal influenza vaccine, with five receiving vaccines within a year of study enrollment.

For the 358 animal-exposed participants, lifetime animal exposure included the following: horses (76.0%), camels (39.4%), goats (55.0%), sheep (49.4%), cattle (48.3%), pigs (15.4%), and domestic poultry (9.5%). The majority (91%)

Table 2. Characteristics of study subjects upon enrollment, Mongolia, 2009. Unadjusted odds ratio for animal-exposed participants compared with non-animal-exposed against control participants with binary logistic regression

Variables	Total N	Exposed N (%)	Controls N (%)	Unadjusted OR (95% CI)
Age (years)				
18–32	148	130 (36.3)	22 (27.2)	1.2 (0.6–2.2)
33–45	139	105 (29.3)	34 (42.0)	0.6 (0.4–1.1)
46–75	152	123 (34.4)	25 (30.9)	Ref
Gender				
Female	210	179 (50.0)	31 (38.3)	1.6 (1.0–2.6)
Male	229	179 (50.0)	50 (61.7)	Ref
Indoor water missing*				
Yes	412	353 (99.2)	59 (72.8)	43.3 (12.4–232.8) ^{†,‡}
No	25	3 (0.8)	22 (27.2)	Ref
Ever received vaccination for human influenza*				
No	411	346 (96.7)	65 (92.9)	2.2 (0.6–7.0) [†]
Yes	17	12 (3.4)	5 (7.1)	Ref
Heart disease, hypertension, or stroke*				
Yes	72	68 (19.0)	4 (6.1)	3.6 (1.3–10.3) ^c
No	352	290 (81.0)	62 (93.4)	Ref
Chronic breathing problems*				
Yes	43	38 (10.6)	5 (7.3)	1.5 (0.6–4.0)
No	384	320 (89.4)	64 (92.8)	Ref
Other chronic medical problems*				
Yes	65	58 (16.3)	7 (10.0)	1.8 (0.8–4.0)
No	361	298 (83.7)	63 (90.0)	Ref
Ever used tobacco products*				
Yes	115	100 (27.9)	15 (22.1)	1.4 (0.7–2.5)
No	311	258 (72.1)	53 (77.9)	Ref
Developed a respiratory illness in the last 12 months*				
Yes	190	159 (44.4)	31 (44.9)	1.0 (0.6–1.6)
No	237	199 (55.6)	38 (55.1)	Ref

*Covariate has some missing data.

[†]Fisher's exact method used.[‡]Statistically significant data ($P < 0.05$).

of the exposed participants reported their animal exposures to have occurred recently (since 2003). Seventy-five participants (17.1%) reported recent disease outbreaks in their horses or camels.

Elevated MN titers against EIV were sparse. Enrollment sera from four participants had elevated antibody titers against A/Equine/Mongolia/01/2008(H3N8; Table 3); of which 3 (75%) had a titer of 1:10 and reported recent occupational exposure to horses. One participant was a 27-year-old male who had been a horse and camel herder for the last 10 years. The other two participants were members of the same family household (47-year-old male and 46-year-old female), and both had been horse herders for the last 18 years. All three participants reported recent disease outbreaks in their equine herds and also farmed cattle,

sheep, and goats. While none reported ever receiving a seasonal influenza vaccine, all three had elevated antibody titers ($\geq 1:40$) against the A/Brisbane/10/2007(H3N2) human influenza virus.

The fourth EqH3N8 seropositive (1:20) participant was a 32-year-old female who worked as a national health official. While she reported working with cows for the last 17 years and pigs for the last 2 years, she reported no exposure to horses or camels. Furthermore, this participant also had an elevated titer (1:10) against the A/Teal/Hong Kong/w312/97 (H6N1) AIV; she was the only seropositive subject. She reported no exposure to poultry or wild birds. She did report receiving a seasonal influenza vaccine in 2006 and was seropositive (1:160) against A/Brisbane/10/2007(H3N2). She reported experiencing 1–2 episodes of a respiratory illness (fever and cough or sore throat) in the 30 days prior to her enrollment and reported 3–5 episodes of respiratory illness among household family members (six adults and two children) within the same time period.

Seroreactivity against the remaining AIV strains was also very sparse. Three participants had elevated titers (1:10) against A/Hong Kong/1073/1999(H9N2). One participant had a prior 4-year history of household exposure to chickens, but none cited recent poultry or wild bird exposure. Only one AvH9N2 seropositive participant was also seropositive against A/Brisbane/10/2007(H3N2), and all three reported never receiving an influenza vaccination.

One participant, a 69-year-old male, had an elevated antibody titer (1:10) against A/Cygnus/Mongolia/3/2009 (H5N1) HPAI virus. This participant was a herder of horses, camels, sheep, goats, and cattle, but he reported no poultry or wild bird exposure. He had never received an influenza vaccine, but he did have an elevated antibody titer (1:80) against A/Brisbane/10/2007(H3N2).

In examining serological reactivity against human influenza virus strains, 287 participants (65.8%) had elevated titers ($\geq 1:40$) against A/Brisbane/10/2007(H3N2). Forty-two participants (9.6%) had elevated titers against A/Brisbane/59/2007(H1N1). Seven participants (1.6%) had elevated titers against the 2009 pandemic influenza virus, A/Mexico/4108/2009(H1N1). Six of the seven were enrolled in May–June 2009, and one participant was enrolled in January 2009.

Discussion

A 1966 report by US National Institutes of Health researchers clearly documented experimental EIV infections in humans.⁶ While 4 (12%) of 33 inoculated subjects experienced clinical illness, 20 subjects (61%) had evidence of infection by an observed antibody titer increase. As Mongolia has large populations of horses that roam its vast plains, comingling with aquatic birds that occasionally introduce HPAI, it seems

Table 3. Distribution of elevated MN titers against equine and avian influenza viruses and HI titers against human influenza among seropositive study participants in Mongolia

Participant	A/Equine/Mongolia /01/2008(H3N8)	A/Cygnus/ Mongolia/ 3/2009(H5N1)	A/Teal/Hong Kong/ w312/97(H6N1)	A/Hong Kong/1073/ 1999(H9N2)	A/Brisbane/10/ 2007(H3N2)
27-year-old male	10	<10	<10	<10	320
47-year-old male	10	<10	<10	<10	40
46-year-old female	10	<10	<10	<10	40
32-year-old female	20	<10	10	<10	160
69-year-old male	<10	10	<10	<10	80
52-year-old male	<10	<10	<10	10	80
63-year-old male	<10	<10	<10	10	<10
23-year-old male	<10	<10	<10	10	<10

MN, Microneutralization assay; HI, Hemagglutination inhibition assay.

prudent to study Mongolia's rural people for evidence of zoonotic influenza virus infection.

Our study data, and human influenza surveillance data in Mongolia, suggest that if AIV or EIV infections occur in Mongolians, they likely occur infrequently. It seems possible that the low MN titers detected might reflect EIV infections during the 2007–2008 H3N8 equine epizootic that had waned¹² by human blood draw in 2009. However, it seems equally plausible that the low tiers may be explained by cross-reactivity from previous human influenza A virus infection, especially that due to human H3N2 virus.

The sparse serological evidence for human EIV and AIV infections in this study population suggests that neither EIV nor AIV are currently posing a significant human health risk to rural Mongolians. Nevertheless, Mongolia with its large populations of horses and migrating birds seems an important place to continue to conduct surveillance for the emergence of novel influenza strains.

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Authorship addendum

Nyamdavaa Khurelbaatar involved in the study design, Mongolia principal investigator, Mongolian study coordination. Whitney S. Krueger involved in IRB approval, study coordination, data management, statistical analysis, and manuscript preparation. Gary L. Heil involved in the laboratory assay design and laboratory analyses. Badarchiin Darmaa managed enrollment and data collection at one site in Mongolia. Daramragchaa Ulziimaa involved in the IRB approval, managed enrollment, and data collection at one site in Mongolia. Damdindorj Tserennorov managed enrollment and data collection at one site in Mongolia. Ariungerel Baterdene involved in the data management and entry in Mongolia. Benjamin D. Anderson prepared data summaries and manuscript. Gregory C. Gray involved in the study design, overall principal investigator, data interpretation, and manuscript preparation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Influenza serological assay methods.