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SHORT REPORT
Serological investigations on West Nile virus in birds and horses in Shanghai, China

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SUMMARY

West Nile virus (WNV) infection is an emerging zoonosis that threatens global public health. In this study, a total of 95 bird serum samples from 14 species and 341 horse serum samples were collected from 2008 to 2010 in Shanghai, China. All serum samples were screened initially for WNV-reactive antibodies using a competitive ELISA. The positive samples detected by ELISA were further confirmed using a plaque-reduction neutralization test (PRNT) for WNV and its most closely related flaviviruses in the area to avoid false positives due to cross-reactivity. Five (5·3%) of the bird serum samples and none (0·0%) of the horse serum samples tested positive for WNV antibodies. The findings strongly suggest that some of the birds, specifically the resident birds in China, had been exposed to WNV.

Key words: Birds, horses, China, West Nile virus, serological investigation.

West Nile virus (WNV) is an emerging arthropod-borne flavivirus that belongs to the Japanese encephalitis virus (JEV) serocomplex. It was first isolated in the West Nile District of Uganda in 1937 and is now prevalent worldwide [1]. Before 1994, WNV infections only occurred sporadically in humans and horses. Since the outbreak of WNV encephalitis in New York City and Louisiana state in the USA in 1999 and 2002, respectively [2–4], susceptible animal species have been identified in increasing numbers, including hundreds of species of birds, horses, reindeer, sheep, white-tailed deer, bears, cats, and dogs [5]. Among these species, certain birds are important reservoir hosts that play dominant roles in WNV transmission. Migrating birds have been implicated in spreading WNV in Europe, Asia, Africa and the Middle East [6]. Surveys on WNV infections in birds can indicate the presence of the virus before human and horse WNV cases appear. Therefore, birds are considered to be one of the most effective early indicators of WNV presence in a specific region [7]. Horses may develop severe encephalitis from WNV infection and are considered dead-end hosts, similar to humans [8]. An increasing number of severe outbreaks in horses has been reported in Europe [9]. Thus, the assessment of WNV activity and the implementation of surveillance programmes in a region are useful. The objective of the present study is to analyse the presence of specific anti-WNV antibodies in birds and horses in Shanghai, China, and to

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Table 1. *Results of serological investigations on WNV in birds in shanghai, China, as determined by ELISA and PRNT*

Species	General age*	Sampling date	Status	Sample no.	Positive no. (by ELISA)	Positive no. (by PRNT)
Aix galericulata (Mandarin duck)	Hatch-year and adult	Aug.–Sept. 2009	Free-ranging migrant	12	2	2
Anas platyrhynchos (Mallard)	Adult	Aug.–Oct. 2009	Free-ranging migrant	10	1	0
Passer montanus (Eurasian tree sparrow)	Hatch-year and adult	Nov.–Dec. 2009	Free-ranging resident	11	0	0
Larus saundersi (Saunders's gull)	Adult	Nov.–Dec. 2009	Free-ranging migrant	12	1	1
Nycticorax nycticorax (Black-crowned night heron)	Hatch-year	Sept. 2009	Captive	3	0	0
Grus japonensis (Red-crowned crane)	Adult	Jan. 2010	Captive	3	1	1
Phoenicopterus roseus (Greater flamingo)	Hatch-year	Oct. 2009	Captive	3	0	0
Pavo muticus (Green peafowl)	Adult	Aug. 2010	Captive	4	1	1
Cygnus atratus (Black swan)	Hatch-year	Jan. 2010	Captive	3	0	0
Anas poecilorhyncha (Chinese spot-billed duck)	Hatch-year and adult	Aug. 2010	Free-ranging resident	4	0	0
Anas formosa (Baikal teal)	Adult	Nov.–Dec. 2009	Free-ranging migrant	6	1	0
Pica pica (Common magpie)	Hatch-year and adult	Aug.–Sept. 2010	Free-ranging resident	10	0	0
Phasianus colchicus (Common pheasant)	Adult	Aug.–Oct. 2010	Free-ranging migrant	11	2	0
Coturnix coturnix (Common quail)	Adult	Dec. 2009	Free-ranging migrant	3	0	0
Total				95	9 (9·5 %) ($P<0·05$)	5 (5·3 %) ($P<0·05$)

WNV, West Nile virus; ELISA, enzyme-linked immunosorbent assay; PRNT, plaque-reduction neutralization test.
* The age of hatch-year is <1 year old; the age of adult is >1 year old and lived through the period of immaturity.

Table 2. *Reciprocal $PRNT_{90}$ results of WNV-positive samples detected by ELISA*

Species (ELISA-positive)	Sample ID	WNV	JEV	BYDV	WNV titre/ JEV titre
Aix galericulata (Mandarin duck)	No. 1	200	20	<10	10
Aix galericulata (Mandarin duck)	No. 2	200	40	<10	5
Anas platyrhynchos (Mallard)	No. 1	<10	100	<10	—
Larus saundersi (Saunders's gull)	No. 1	400	10	<10	40
Grus japonensis (Red-crowned crane)	No. 1	200	<10	<10	—
Pavo muticus (Green peafowl)	No. 1	200	<10	<10	—
Anas formosa (Baikal teal)	No. 1	<10	100	<10	—
Phasianus colchicus (Common pheasant)	No. 1	<10	100	<10	—
Phasianus colchicus (Common pheasant)	No. 2	<10	200	<10	—
Horse	No. 1	<10	200	—	—
Horse	No. 2	<10	50	—	—
Horse	No. 3	<10	100	—	—
Horse	No. 4	<10	100	—	—
Horse	No. 5	<10	40	—	—
Horse	No. 6	<10	200	—	—

PRNT, Plaque-reduction neutralization test; WNV, West Nile virus; ELISA, enzyme-linked immunosorbent assay; JEV, Japanese encephalitis virus; BYDV, Baiyangdian virus.

provide valuable information on WNV transmission in the region.

In this study, a total of 95 bird serum samples from 14 species and 341 horse serum samples were collected by the Shanghai Animal Disease Control Centre from 2008 to 2010. The 95 bird serum samples were collected from four districts (Minhang, Jinshan, Jiuduansha, Wuku) around Shanghai and blood was typically collected through jugular venepuncture. The dates of collection were between late summer and winter from 2009 to 2010, coinciding with the period of southern migration of wild birds (August–October) and during the time that winter visitors are present (around November–January) (Table 1). The 341 horse serum samples were collected from seven districts (Fengxian, Qingpu, New Pudong, Minhang, Jinshan, Songjiang, Chongming) around Shanghai from 2008 to 2010. None of the animals had a history of WNV vaccination. None of the birds and horses showed obvious clinical symptoms of the disease. All serum samples were screened initially for WNV-reactive antibodies via competitive enzyme-linked immunosorbent assay (ELISA) using commercial kits (ID VET, France). Data are expressed as relative percentages, and inhibition values $\geqslant 50\%$ are indicative of the presence of viral antibodies. The positive samples detected by ELISA were further confirmed using a plaque-reduction neutralization test (PRNT) with WNV strain NY99-4132 [10]. To avoid false positives due to cross-reactivity, the positive samples were also screened for neutralizing antibodies against JEV (strain 131V), four serotypes of dengue virus (DENV, strains ZJ01/2004, FJ-10, 07CHLS001, Guangzhou B5), and Baiyangdian virus (BYDV, strain BYD-1), which are flavivirusesprevalent in the area. Titres are expressed as the reciprocals of serum dilution yielding a 90% reduction in the number of plaques ($PRNT_{90}$). Samples with reciprocal $PRNT_{90}$ titres >10 were considered positive for flavivirus infection. A fourfold or greater difference in the antibody titres was required for a specific flavivirus to be considered an aetiological agent of the infection.

Of the 95 bird serum samples from 14 species, nine (9·5%) serum samples were positive for WNV antibodies, as determined using ELISA. When the nine ELISA-positive samples were tested by PRNT, five (5·3%) were positive for WNV antibodies, and negative for JEV and DENV ($PRNT_{90}$ titre <10), whereas the other four ELISA-positive bird serum samples were positive for JEV (Tables 1, 2). Of the 14 species, four (Mandarin duck, Saunders's gull, Red-crowned crane, Green peafowl) were positive for WNV antibodies (Tables 1, 2). All the seropositive birds were aged >1 year, had lived through the period of immaturity, and were at the adult stage (Table 1). Of the five WNV-positive bird serum samples, three were collected from migratory birds (Mandarin duck, Saunders's gull) in Jiuduansha (a sand island that serves as an ideal and important stopover point for

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migrating birds near Shanghai). Each year, a variety of migratory birds fly to Shanghai wintering in or passing through Shanghai during migration [11–13]. However, migratory birds travel across many habitats and regions, increasing their chances of exposure to a variety of pathogens. Moreover, due to lack of further examination of the virus and failure to check whether antibody was caused by recent infections, the presence of WNV antibodies in some adult migratory birds is not sufficient to determine the precise location of infection and the infection transmission route. The other two WNV-positive bird serum samples (Red-crowned crane, Green peafowl) were collected from a zoo. The two positive birds were adult birds, born in captivity, with no travel history. Moreover, very few interactions occur between the zoo and the outside world; hence, the birds may have acquired their infections locally. The infections may be related to the unique lifestyle of the birds, who like wet and water environments, thereby increasing the risk of mosquito exposure [14]. Since April 2010, a severe outbreak of duck BYDV (a Tembusu-like flavivirus) viral infection, which is accompanied by egg drop, a decline in feed uptake, and ovary-oviduct disease, has spread around the major duck-producing regions in China [15]. In this study, the BYDV antibody titres were further tested in WNV-positive sera by taking into account the BYDV, which may confuse the serological results. The results showed that none was positive for the BYDV ($PRNT_{90}$ titre <10) (Table 2). Therefore, our results were not affected by the BYDV infection. Considering the limitations on the number of bird serum samples from each species, the seroprevalence of WNV antibodies may not be highly representative of WNV infection status in birds in China. However, we have demonstrated the presence of WNV infection in birds in Shanghai, especially in two resident birds that were infected locally.

Of the 341 tested horse serum samples, six (1·8%) were positive for WNV antibodies, as determined by ELISA. None (0·0%) of these ELISA-positive samples was positive for WNV antibodies when they were further tested using PRNT ($PRNT_{90}$ titre <10). The six serum samples were also negative for DENV, but all were positive for JEV (Table 2). The results showed little evidence of an active WNV infection in horses in Shanghai. However, continued surveillance of horses may enable prompt detection of WNV in the region.

To the best of our knowledge, this study is the first to report on the seroprevalence of WNV in birds and horses in China. Despite the lack of confirmed cases of WNV-attributed diseases in China, experimental studies have demonstrated that *Culex* mosquitoes in China sustain WNV, and are competent laboratory vectors of WNV [16]. The current study demonstrates that WNV-positive antibodies are present in birds (5·3%) but absent in horses (0·0%). Considering that Shanghai is a densely populated centre where susceptible vectors live, the risk of a future WNV epidemic warrants serious consideration. The findings of this study indicate the potential danger of locally acquired WNV infection, represented by the two seropositive resident birds. Therefore, the prevalence and spread of WNV in the region should be monitored diligently.

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DECLARATION OF INTEREST

None.

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