

NOTE

Serological survey of equine viral diseases in Mongolia

Ochir Pagamjav^{1,2}, Keiko Kobayashi¹, Hironobu Murakami¹, Yuji Tabata¹, Yasuo Miura¹, Bazartseren Boldbaatar^{1,2} and Hiroshi Sentsui¹

¹School of Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan, and ²Laboratory of Virology, Institute of Veterinary Medicine, Zaisan, 210553, Ulan Bator -11, Mongolia

ABSTRACT

Three hundred sera were collected from horses in various parts of Mongolia in 2007 and seroepidemiological surveys for several equine viruses performed on them. Equid herpesvirus 1 and equine rhinitis A virus were prevalent, and equine arteritis virus and equid herpesvirus 3 were detected over a wide area though their rates of antibody-positivity were not high. Equine infectious anemia was distributed locally. The rates of horses antibody-positive for Japanese encephalitis virus and equine influenza virus were low, but these were detected. Bovine coronavirus antibodies were detected at a high rate, but it was not clear whether they were due to horse coronavirus.

Key words epidemiology, equine virus, horse, Mongolia.

Mongolia has a continental climate with freezing dry weather throughout the winter and a relatively short summer with limited rainfall. The foundation of the economy is the livestock sector, which is based mainly on extensive production of sheep, goats, cattle, horses and camels on pastures. The system for breeding domestic animals in Mongolia is very different to that in other countries. Since animal husbandry is based on a nomadic lifestyle, domestic and wild animals are frequently in both direct and indirect contact, the latter because they share the same pastures and water sources. Thus cross infection can easily occur, not only among horses, but also between horses and other animals.

The horse is one of the most important domestic animal species in Mongolia with a total population of 2.2 million according to the National Statistical Office of Mongolia (Ministry of Food, Agriculture and Light Industry of Mongolia, 2009). Epidemiological studies of equine infec-

tious diseases such as leptospirosis, trypanosomiasis and babesiosis in Mongolian horses have recently been published (1–4). However, no epidemiological investigation of viral diseases among the horses in Mongolia has yet been reported in international journals. In this report, we focused on an epidemiological survey of the viral infectious diseases that are suspected of affecting horses in Mongolia and that can cause them serious health problems; the diseases that could result in economic burdens.

Two groups of horse sera were tested. The sera of the first group (200 samples) were randomly collected from north Selenge province. The 100 samples in the second group of sera were randomly collected from the areas of Tov, Arkhangai, Dornod, Khentii, Dundgobi, Sukhbaatar and Uvs provinces (Fig. 1). Three samples from Uvs province were removed from this group and reclassified as Tuva because the horses had been imported from Tuva in Russia. All serum samples were collected from horses with no

Correspondence

Hiroshi Sentsui, School of Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan.
Tel: +81 466 84 3384; email: sentsui@brs.nihon-u.ac.jp

Received 12 September 2010; revised 22 December 2010; accepted 5 January 2011.

List of Abbreviations: AGID, agar gel immunodiffusion testing; BCoV, bovine coronavirus; CPE, cytopathic effect; EAV, equine arteritis virus; ECoV, equine coronavirus; eFLUAV, equine influenza virus; EHV, equid herpesvirus; EIA-ED, equine dermal cells persistently infected with equine infectious anemia virus; EIAV, equine infectious anemia virus; EMEM, Eagle's minimum essential medium; ERAV, equine rhinitis A virus; HA, hemagglutinin; HI, hemagglutination inhibition; JEV, Japanese encephalitis virus; OIE manual, the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals issued by Office International des Epizooties; TPB, tryptose phosphate broth; VN, virus neutralization.

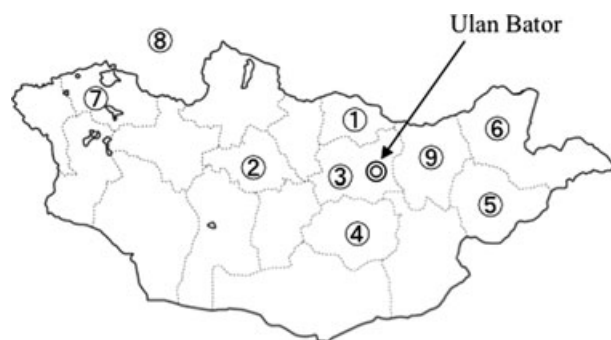


Fig. 1. Map of Mongolia showing the areas in which the horse sera were collected. The provinces are labeled as follows: ① Selenge, ② Arkhangai, ③ Tov, ④ Dundgobi, ⑤ Sukhbaatar, ⑥ Dornod, ⑦ Uvs, ⑧ Tuva (Russia), ⑨ Khentii.

clinical abnormalities. After collection, they were heat inactivated at 56°C for 30 min and exported to Japan (animal quarantine inspection number NFIB070602-011).

Four cell lines and primary horse kidney cells were used for the VN tests. The cells used for each virus are shown in Table 1. RK-13 cells originating from rabbit kidney, HmLu cells from hamster lung and HRT-18 cells from human large bowel cancer were cultivated with EMEM supplemented with 5% FCS and 0.3% TPB. FHK-78 cells originating from horse kidney (5) and primary horse kidney cells were cultivated with EMEM supplemented with 10% FCS. All culture media were supplemented with 100 units/mL penicillin and 100 µg/mL streptomycin. Primary horse kidney cells were prepared by standard tissue culture methods and used within seven passages.

Equine arteritis virus, eFLUAV, EIAV, JEV, EHV-1, EHV-3, ERAV, ECoV and BCoV were used for the epidemiological surveys. Virus strains, cultivated cells and serological tests used for the epidemiological surveys are shown in Table 1.

For the VN test, the sera were titrated in two duplicate wells across a microtiter plate from an initial 1:4 dilution. Then 0.05 mL samples of serial 2-fold serum dilutions were mixed with an equal volume of virus having 200 TCID₅₀/0.1 mL in 96-well microplates and incubated at 37°C for 60 min. Cell suspensions were added to each well and incubated at 37°C until a CPE appeared in the virus control cells. The VN antibody titer was expressed as the reciprocal of the serum dilution that inhibited CPE. Samples that were positive for antibodies at a dilution of 1:4 or greater were considered seropositive.

For the preparation of EIAV antigen for the AGID, EIA-ED were cultivated in medium consisting of 10% FCS and 90% EMEM (6). The cell culture supernatant of EIA-ED was concentrated using polyethylene glycol, treated with ether and used as an antigen. The procedure used for the antigen preparation for AGID is described in the OIE

Table 1. Viruses, cultivated cells and serological tests used in the epidemiological surveys

Virus	Strain	Cell	Serological test used	Reference
EAV	Bucyrus	RK13	VN test	(7)
EIAV	Wyoming	Equine dermal cell	AGID test	(6)
JEV	JaGAR#01	HmLu	VN test	(8)
eFLUAV	A/eq/LaPlata/93	Chick embryo	HI test	
ECoV	NC99	HRT-18	VN test	(9)
BCoV	Kakegawa	HRT-18	VN test	(10)
EHV-1	HH-1	FHK78	VN test	(11)
EHV-3	1118	HK	VN test	(12)
ERAV	98C11	RK13	VN test	(13)

manual. AGID tests were also performed according to the OIE manual.

Hemagglutinin antigen for eFLUAV was purchased from Nisseiken (Tokyo, Japan). HI tests were performed according to the manufacturer's instruction. The HI titer was expressed as a reciprocal of the serum dilution that inhibited HA activity. Serum that showed an HI titer of 8 or more was classified as positive.

Equine arteritis virus infection was prevalent among horses in most provinces in Mongolia. Positive reactors were found in many provinces, 45 (15.0%) of the 300 horse sera being positive, but none were detected in Dornod and Khentii provinces (Table 2), possibly because there were few test sera from these provinces. EAV cannot be differentiated clinically from a number of other respiratory and systemic equine viral diseases such as equine influenza, EHV-1 and 4 and equine adenovirus infections (7, 14). The disease also has clinical similarities to toxicosis caused by hoary alyssum. Therefore, outbreaks of this disease have been misdiagnosed as other illnesses and not correctly identified as EAV infections. It is not clear whether the virus originated in Mongolia or came from Europe. Since many horses were sent from Mongolia to Europe during World War II, some of them might have been infected with the virus there and subsequently taken it back to Mongolia. If, in the future, EAV is again isolated from a horse in Mongolia its origin will become clear genetically or serologically. This is the first report of the presence of EAV among horses in Mongolia.

Antibodies to eFLUAV were found in 17 (5.7%) of the 300 horses (Table 2). The present serological studies were done using the H3N8 type (A/equine/La Plata/93[H3N8]) and the antibody-positive horses were detected only in Selenge province. It is not certain whether this discrepancy between Selenge and the other provinces is due to natural infection or vaccination. No information about vaccination of the horses from which sera were collected was available. There was a big outbreak of equine influenza in

Table 2. Antibodies to equine viruses in Mongolian horse sera

Province	Positive sera/tested sera						
	EAV†	eFLUAV‡	EIAV§	JEV¶	EHV-1*	EHV-3**	ERAV***
Selenge	19/200	17/200	49/200	11/200	109/189	42/200	12/193
Arkhangai	1/12	0/12	0/12	1/12	10/12	9/12	10/12
Tov	9/42	0/42	2/42	0/42	31/36	9/37	36/41
Dundgobi	8/18	0/18	0/18	0/18	11/11	3/11	16/18
Sukhbaatar	2/6	0/6	0/6	1/6	6/6	0/6	6/6
Dornod	0/3	0/3	0/3	0/3	3/3	1/3	3/3
Uvs	6/15	0/15	0/15	0/15	15/15	1/15	13/15
Tuva (Russia)	0/3	0/3	0/3	0/3	3/3	1/3	3/3
Khentii	0/1	0/1	0/1	0/1	1/1	0/1	1/1
Total (%)	45/300 (15.0)	17/300 (5.7)	51/300 (17.0)	13/300 (4.3)	189/276 (68.5)	66/288 (22.9)	100/292 (34.2)

† EAV detected by VN test; ‡eFLUAV detected by HI test; §EIAV detected by AGID test; ¶JEV detected by VN test; *EHV-1 detected by VN test; **EHV-3 detected by VN test; ***ERAV detected by VN test.

Mongolia from the autumn of 2007 (15). The horse sera studied in this serological survey were collected by March 2007. The low rate of antibody-positivity present at that time would have been an important factor in the later epidemic of the disease.

According to the records of the Mongolian government, equine infectious anemia was first diagnosed in 1952 (16), and antibody-positive horses were detected in a recent study (17). However, the prevalence of the disease is not clear. In the present study, the seroprevalence of EIAV was considerably greater in Selenge province (24.5%). This may indicate that the humid climate in Selenge supports the survival of bloodsucking insects. The horse population in Selenge is also bigger than in other provinces.

Antibody to JEV was also detected, though the rate of positivity was very low (it was found in only three provinces). The breeding of pigs, a carrier of JEV (18), is not common in Mongolia, however wild boars do live

there. Therefore, the conditions for an epidemic of JEV are insufficient. Furthermore, the seropositive horses did not have high antibody titers to JEV. West Nile virus, which cross-reacts with JEV, is present in Siberia (19). It remains to be clarified in the future whether the JEV antibody-positivity detected in horses in this study was due to JEV infection or to other viruses with an antigen in common with JEV, such as West Nile virus.

Antibodies for EHV-1 and ERAV were detected all over Mongolia, but the positivity rate was lower in Selenge province than in other places. This was probably because the blood samples were collected mainly from young horses. EHV-3 antibody-positive horses were detected in most provinces, though the positivity rate was not as high as for EHV-1 (Table 2). Horse reproduction in Mongolia occurs through natural mating and the rate of artificial insemination is low. This could be one of factors responsible for so many horses being antibody positive for EHV-3.

Antibodies to coronaviruses were looked for in 22 sera randomly selected from the 300 samples. Nineteen horses were positive for the BCoV Kakegawa strain, but all were negative for ECoV strain NC99 (Table 3). Several coronavirus genotypes have been isolated in cattle (20). Because horses and cattle share the same pastures and water sources on farms in Mongolia, it is not clear whether the horses were infected with BCoV or ECoV.

The present study provides information on the current prevalence of representative equine viral diseases in Mongolia. To elucidate the epidemiology of these viruses and control horse diseases in Mongolia, further more comprehensive epidemiological investigations are required.

ACKNOWLEDGMENTS

We would like to thank Mr. Kim Barrymore for critical reading of the manuscript. This work was supported in

Table 3. Antibodies to coronaviruses in 22 randomly selected horse sera

Horse No.	Antibody to		Horse No.	Antibody to	
	ECoV†	BCoV‡		ECoV	BCoV
4	<4§	64	204	<4	8
5	<4	128	206	<4	16
12	<4	8	207	<4	4
13	<4	16	208	<4	<4
43	<4	32	209	<4	16
46	<4	8	220	<4	<4
47	<4	16	224	<4	4
48	<4	64	233	<4	8
56	<4	16	248	<4	<4
113	<4	16	288	<4	4
202	<4	32	300	<4	4

†ECoV NC99; ‡BCoV Kakegawa strain; §antibody was detected by VN test.

part by a Grant-in-Aid for the Academic Frontier Project for Private Universities from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the fellowship fund of the Regional Research Institute of Agriculture in the Pacific of Nihon University.

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