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Molecular epidemiological survey and genetic characterization of *ovine gammaherpesvirus-2* in Mongolian livestock

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ABSTRACT. Sheep-associated malignant catarrhal fever (SA-MCF), caused by *ovine gammaherpesvirus-2* (OvHV-2), is a fatal disease in all ruminants. The epidemiological survey and molecular characterization of OvHV-2 in Mongolian livestock were performed. Of 928 blood samples, 14 were positive for OvHV-2 in sheep and native cattle from Tsenkher County and in sheep from Lun County. Phylogenetic analyses revealed that the tegument gene of OvHV-2 sequences from Mongolian animals is identical to that in animals from Egypt, India, and Turkey, and is 98.0% similar to that in animals from Germany and Brazil. To our knowledge, this is the first confirmed report of OvHV-2 in Mongolian livestock, and could provide useful information for controlling SA-MCF.

KEY WORDS: Mongolian livestock, sheep-associated malignant catarrhal fever, tegument protein gene

Malignant catarrhal fever (MCF) is a serious and usually fatal disease in domestic and wild ruminants that is characterized by low morbidity and high mortality [7]. Its causative agents are herpes viruses in the MCF virus group, belonging to the genus *Macavirus* in the subfamily *Gammaherpesvirinae* [3]. Six out of ten identified members of the MCF virus group are associated with the clinical disease in natural conditions. Of these, *alcelaphineherpesvirus-1* (A1HV1) and *ovine gammaherpesvirus-2* (OvHV-2) are the major causative agents, and are responsible for wildebeest-associated MCF (WA-MCF) and sheep-associated MCF (SA-MCF) [9]. These viruses, resulting in inapparent infection in their respective natural hosts, could cause fatal lympho-proliferative disease when they infect other susceptible hosts [10]. Although preliminary diagnoses of SA-MCF have been reported in Mongolian cattle [11], the diagnoses have not been confirmed with molecular diagnostic assays such as polymerase chain reaction (PCR) or sequencing analysis. In the present study, a molecular survey and genetic characterization of OvHV-2 based on the tegument protein gene were performed in samples from cattle, yaks, sheep, and goats in five different Mongolian areas.

A total of 928 whole blood samples were collected from randomly selected free-range livestock, including native cattle (n=117), yaks (n=100), dairy breed cattle (Holstein, Simmental, and Alatau) (n=300), sheep (n=211), and goats (n=200) from 5 different Mongolian sampling sites in 2014. Genomic DNA was extracted from blood samples using a Genomic DNA Purification Kit (Promega Corp., Madison, WI, U.S.A.), according to the manufacturer's instructions. The β-globin gene was amplified as an internal control to confirm the presence of DNA in the templates according to a previous report [12]. The samples were screened for the tegument protein gene of OvHV-2 (238 bp) with a semi-nested PCR assay using 1.5 μl of extracted sample DNA and 28.5 μl of reaction mixture [1]. Further, positive samples were subjected to sequencing analysis by methods described previously [8].

Our results revealed that of 928 samples screened, the following 14 were positive for OvHV-2: 9 of 10 sheep (90.0%) and 2 of 20 native cattle (10.0%) samples from Tsenkher County in Arkhangai Province, and 3 of 201 sheep (1.5%) samples from Lun County in Tuv Province. In addition, all positive sheep samples belonged to adults, and the two positive native cattle samples belonged to 7- and 11-year-old females (Table 1). Thus, OvHV-2 was detected from both the sheep and native cattle in Tsenkher County of Arkhangai Province, where SA-MCF infection has historically been reported from native cattle [11]; native cattle and sheep were kept in the same farm in the Mongolian livestock herding style. In contrast, there has been no report of SA-MCF infection in Lun County Tuv Province, which is another positive sampling area. These two areas are approximately 350 km apart, with no correlation between disease incidence and animal movement existing between these areas; animals from these sampling sites may have contracted the infectious diseases independently.

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Table 1. OvHV-2 detection in Mongolian sheep and cattle

Province/city	County/district	Animal species	Positive/tested animals (%)	Positive sample ID	Age	Sex	Accession number
Arkhangai	Tsenkher	Sheep	9/10 (90.0)	1	Adult	F	LC203437
				2	ND	M	
				3	ND	F	
				4	ND	F	
				5	ND	F	
				6	ND	F	
				7	ND	F	
				8	ND	M	
				9	ND	F	
		Native cattle	2/20 (10.0)	8	7 Y	F	
				18	11 Y	F	
	Bulgan	Yaks	0/72	-	-	-	
Tuv	Lun	Sheep	3/201 (1.5)	34	2 Y	M	
				40	2 Y	M	
				67	3 Y	M	
	Bornuur	Cattle	0/97	-		-	
		Goats	0/200	-		-	
		Dairy breed cattle	0/96	-		-	
Ulaanbaatar	Songinokhairkhan	Yaks	0/28	-		-	
		Dairy breed cattle	0/204	-		-	

Y: years, M: male, F: female, ND: not determined.

In addition, all 14 samples positive for the tegument gene of OvHV-2 were subjected to sequencing analysis; they aligned with 100% sequence identity. Therefore, one representative sequence was deposited at DDBJ with the accession number LC203437. The BLAST searching tool showed that most overlapping sequences originated from Brazil, Turkey, Egypt, Germany, and India, while none of the sequences originated from China or Russia, which are countries bordering Mongolia. There was not much diversity among similar sequences, with the Mongolian sequence exhibiting 100% similarity with sequences from Egypt (KP015737; JF832385), India (KJ020269), and Turkey (JN084011), and approximately 98.0% similarity with other phylogenetic branch sequences from Germany and Brazil (Fig. 1).

Our findings indicate that Mongolian sheep are the primary hosts for OvHV-2 and that this pathogen may be widely prevalent in the sheep population. Infected sheep are known to be a major source of OvHV-2; the infection is transmitted horizontally between animals in close contact with each other [6]. Under natural flocking conditions, most lambs remain uninfected until they are at least 2 months old [5]; they could remain uninfected as adults by avoiding contact with infected sheep from an early age [4]. Sheep producers and zoos in America and Europe use this strategy to maintain OvHV-2 free sheep populations [2]. However, Mongolian herders conduct traditional livestock herding practices, where the cattle are kept in the same backyard or pasture as sheep, goats, etc.; there is a high potential risk of disease outbreak in cattle or other susceptible hosts in areas with high pathogen prevalence.

This study represents the first attempt at a detailed investigation of OvHV-2 in Mongolian livestock. Further studies are required to determine the prevalence of the infection in other Mongolian areas and to identify the genetic diversity of the pathogen between regions, for developing strategies to control the disease in this country.

CONFLICT OF INTEREST. The authors declare that they have no conflicts of interest.

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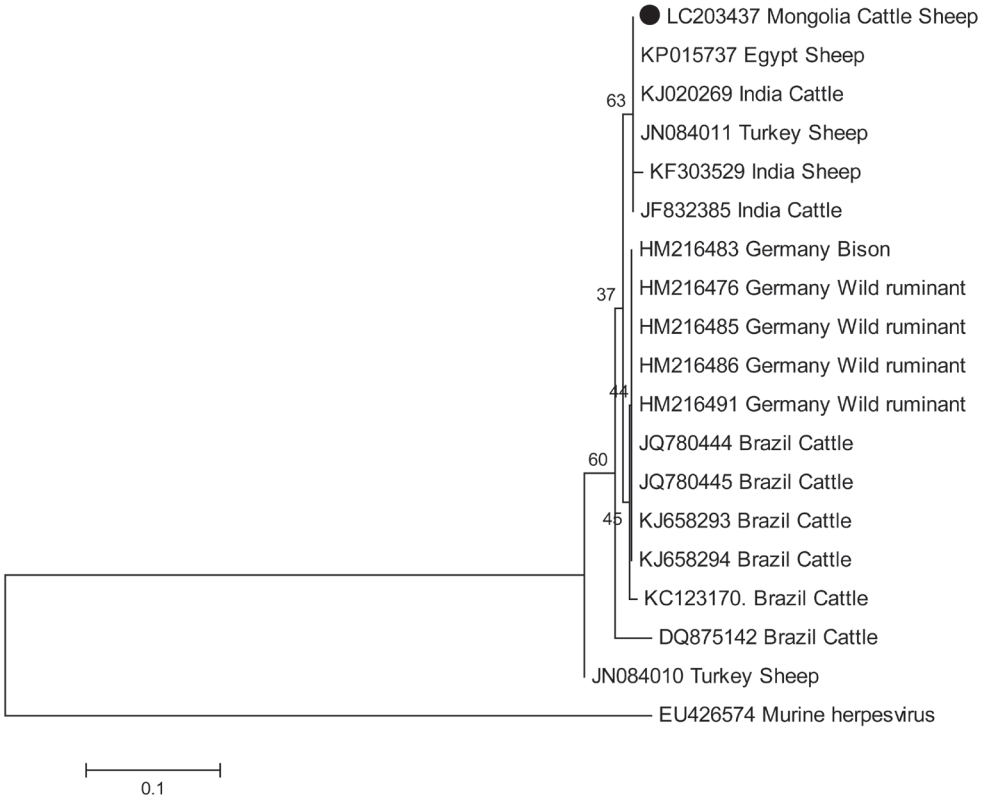

Fig. 1. Phylogenetic relationships of OvHV-2 based on the tegument protein gene. OvHV-2 is the etiological agent of sheep-associated malignant catarrhal fever (SA-MCF). Sequences derived from the present study are indicated by bullets. The tree was constructed using the neighbor-joining method and was supported by 1000 bootstrap replications. It shows the relationship between a 238-bp segment of the tegument protein gene of OvHV-2 obtained in this study and other related sequences from GenBank.

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