Short Communication

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Evidence of two genetically different lymphotropic herpesviruses present among red deer, sambar, and milu herds in China

Hongwei Zhu[1], Huitao Liu[2], Xin Yu[1], Jianlong Zhang[1], Linlin Jiang[1], Guozhong Chen[1], Zhibin Feng[1], Youzhi Li[3], Tao Feng[3], Xingxiao Zhang[1,*]

[1]*School of Life Sciences, Ludong University, Yantai 264025, China*
[2]*Institute of Special Economic Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun 130112, China*
[3]*Shandong Provincial Key Laboratory of Quality Safety Monitoring and Risk Assessment for Animal Products, Jinan 250022, China*

Herpesvirus infections in *Cervidae* are a serious threat affecting some deer species worldwide. In our attempt to identify malignant catarrhal fever-associated herpesviruses in deer herds, ten gammaherpesviral DNA fragments were identified in five species of deer in herds in China by using a pan-herpesvirus polymerase chain reaction assay targeting viral DNA polymerase. Notably, in sambar (*Rusa unicolor*), a novel gamma-2 herpesvirus was identified that showed a close relationship with fallow deer lymphotropic herpesvirus (LHV), while the other fragments were phylogenetically grouped together with Elk-LHV. Determination of whether these viruses have any clinical implication in these deer species should be undertaken urgently.

Keywords: deer species, gammaherpesvirus, phylogenetic analysis, sambar herpesvirus 1

Herpesviridae is a large family of DNA viruses with a variety of hosts and is subdivided into three subfamilies *Alpha-*, *Beta-*, and *Gammaherpesvirinae* as suggested by composite phylogenetic tree analysis using eight core genes [15]. Generally, gammaherpesviruses (γ-HVs) establish latent infections within hosts for the duration of their life, causing diseases in immunosuppressed individuals [2]. Initially, *Gammaherpesvirinae* contained two genera of lymphotropic herpesvirus (LHV), *Lymphocryptovirus* and *Rhadinovirus*, as indicated by phylogenies targeting herpesvirus DNA polymerase (DPOL) gene sequences [8,11]. Subsequently, more γ-HVs were reported, and, as a result, two other genera *Percavirus* and *Macavirus* were included within the subfamily [9]. However, there is a multifurcated clade whose branching details remain unresolved [5].

Ruminant rhadinoviruses (RuRVs) are a subgroup of γ-HVs within the genus *Rhadinoviridae* or gamma-2 herpesvirus [3,8]. Viruses in this subgroup infect ruminant animals, causing subclinical infection or clinically apparent malignant catarrhal fever (MCF) syndrome, a lymphoproliferative disease with high mortality in bison and cattle, and some are incurable in susceptible deer species such as sika deer (*Cervus nippon*) [3]. RuRVs are usually difficult to propagate *in vitro* with the exception of the alcelaphine herpesvirus 1 (AlHV-1), the causative agent for wildebeest-associated MCF. Pan-herpes consensus polymerase chain reaction (PCR) assay for detection of herpesviral DPOL has facilitated the identification of novel γ-HVs. To date, more than 20 rhadinoviruses have been documented in ruminant species. Eight have been identified in deer as a natural host, among which, only MCF virus (MCFV)-WTD is reported to develop MCF syndrome in white-tailed deer (WTD) based on clinical signs and histopathological lesion observations [7,8,10]. Another seven are phylogenetical members of a non-MCF subgroup and have no clinical indications. In this short report, we report on the detection of γ-HV DNA in peripheral blood lymphocytes (PBLs) of five species of deer in herds in China. Notably, a novel γ-HV that had been preliminarily identified in sambar. In addition, we also found that a previously identified elk γ-HV (Elk-LHV) might be prevalent among other deer species, at least among elk, red deer, sambar, and milu.

Herds of five deer species (sambar, Eld's deer, milu, red deer,

Table 1. Deer herd information and gammaherpesvirus infection rate of each herd

Deer species	Geographic location in China	Collection time (mo/d/yr)	Positive rate, % (n)
Red deer (*Cervus elaphus*)	Baotou, Inner Mongolia	10/30/2016	18.2 (4/22)
Sambar (*Rusa unicolor*)	Tunchang, Hainan	2/26/2016	29.4 (5/17)
Milu (*Elaphurus davidianus*)	Dafeng NRC, Yancheng, Jiangsu	12/31/2015	4.3 (1/23)
Eld's deer (*Panolia eldii*)	Datian NRC, Dongfang, Hainan	10/20/2016	0 (0/25)
Reindeer (*Rangifer tarandus*)	Genhe, Inner Mongolia	6/26/2015	0 (0/29)

NRC, Nature Reserve of China.
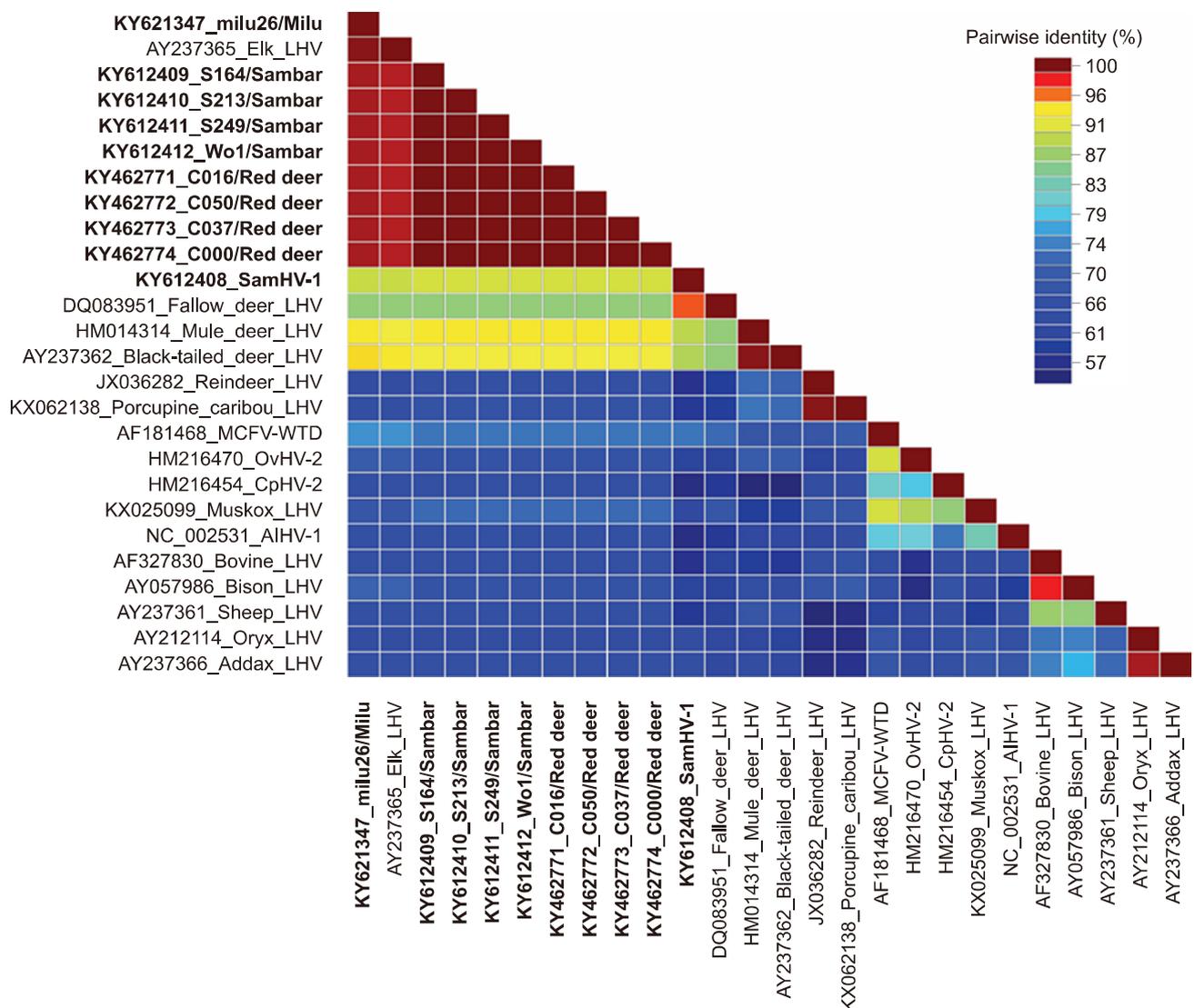
Fig. 1. Pairwise identity matrix of 26 amino acid sequences of gammaherpesviruses DNA polymerase fragments. The sequence alignment was carried out by using the Clustal-Omega program and was visualized via a color-coded matrix. The color in each cell represents a percentage identity score between two sequences as indicated by the color legend in the top right of Fig. 1. GenBank accessions, virus (sample) names and/or hosts are indicated. Viruses identified in this study are shown in bold font. LHV, lymphotropic herpesvirus; MCFV, malignant catarrhal fever virus; WTD, white-tailed deer; OvHV-2, ovine herpesvirus 2; CpHV-2, caprine herpesvirus 2; AIHV-1, alcelaphine herpesvirus 1.

and reindeer) were examined for the presence of LHV. Among these herds, sambar and Eld's deer are distributed in the Tunchang Nature Reserve of China (NRC) and the Datian NRC, Hainan province, respectively. Milu deer in Jilin province were screened as soon as they were introduced from the Dafeng NRC, Jiangsu province. Red deer and reindeer herds are distributed in Baotou and Genhe, respectively, in the Inner Mongolia Autonomous Region, northern China (Table 1).

EDTA-anticoagulated blood samples were collected by a qualified veterinarian present in the farm or nature reserve. For the nucleic acid test, DNA samples extracted from the PBLs were subjected to two-round nested PCR amplification for DPOL by using five degenerate consensus primers as described previously [14]. The resultant sequences of the obtained amplicons, approximate 228 bp in length, were deposited in GenBank (National Center for Biotechnology Information [NCBI], USA). Finally, sequence alignment and phylogenetic analysis were performed with two datasets comprised of the DPOL sequences in this study and those retrieved from the NCBI. Briefly, Pearson-formatted sequences were aligned by using the Clustal-Omega sequence alignment program [13].

The result was then visualized by using the Sequence Demarcation Tool ver. 1.2 (SDTv1.2) [12], which allows the classification of viral sequences based on a color-coded matrix of pairwise similarity scores. For phylogenetic analysis, an unrooted tree was constructed using the maximum likelihood method implemented in PhyML software (ver. 3.0) [6], with the JTT+G substitution model, according to the Akaike's Information Criterion scores, inferred by ProtTest-2.4 [1].

In total, 10 γ-HVs DPOL gene fragments were amplified and identified in 116 individuals of five deer species: 22 red deer, 17 sambar, 23 milu, 25 Eld's deer, and 29 reindeer. The overall rate of infection in these deer herds was 8.6% (10/116). The species-specific infection rates were 18.2% for red deer, 29.4% for sambar, and 4.3% for milu. Using this method, no infections were detected in Eld's deer or reindeer (Table 1).

The γ-HV gene fragments obtained from sambar, red deer, and milu were compared and submitted to GenBank under the ascension numbers KY612408 to KY612412, KY462771 to KY462774 and KY621347, respectively. Sequence alignment showed that a fragment from sambar (sample ID: Lo-3) shared only 80.2% to 81.4% pairwise similarities to those of 25 other
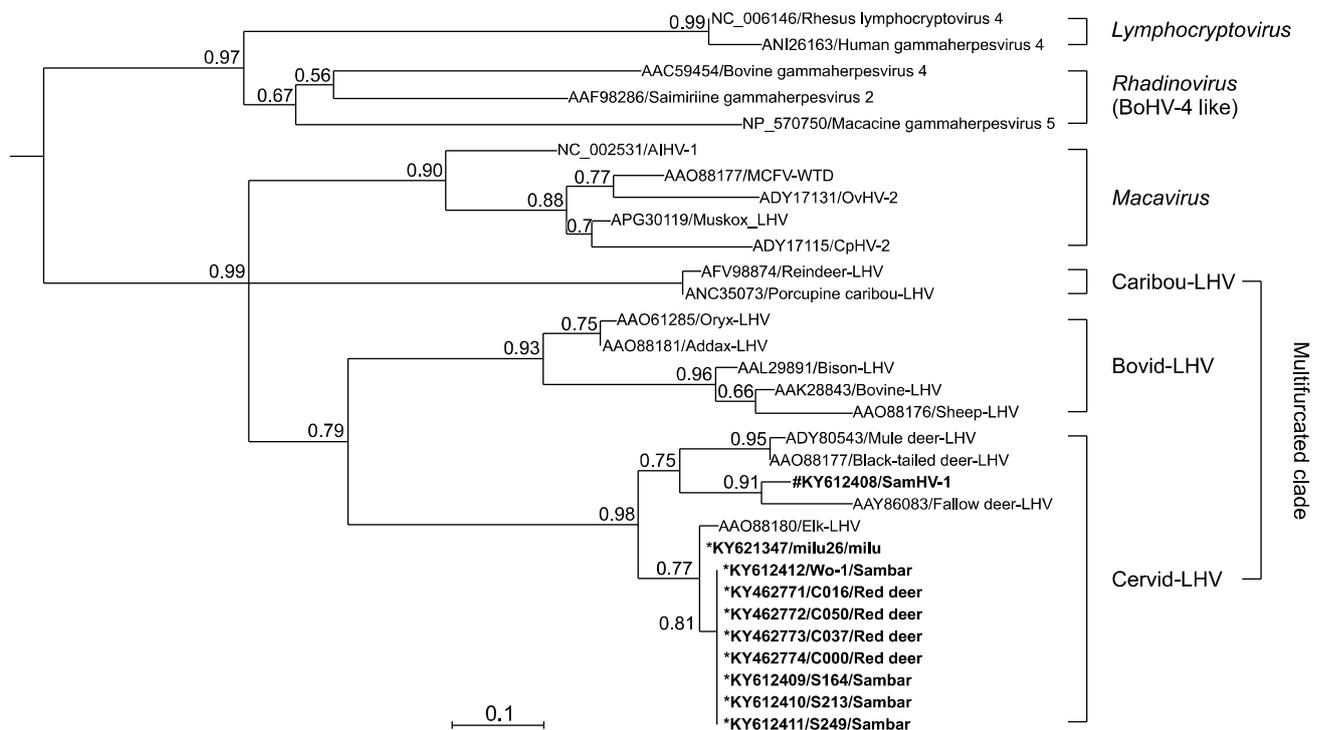
Fig. 2. Phylogram of 31 gammaherpesviruses based on the DNA polymerase fragments. The phylogenetic tree was constructed by using PhyML software (ver. 3.0) [6] with the JTT + G amino acid substitution model. Reliability of the tree was inferred by applying an approximate likelihood ratio test (aLRT). The aLRT values less than 0.50 were collapsed. GenBank accession, virus (sample) name and/or host are indicated in the taxon. Viruses identified in this study are shown in bold font and marked with an asterisk (*), exception for SamHV-1, which is marked with an octothorpe (#). The scale bar indicates the number of amino acid substitutions per site. BoHV-4, bovine gammaherpesvirus 4; AIHV-1, alcelaphine herpesvirus 1; MCFV, malignant catarrhal fever virus; WTD, white-tailed deer; OvHV-2, ovine herpesvirus 2; LHV, lymphotropic herpesvirus; CpHV-2, caprine herpesvirus 2.

viruses or samples, indicating that Lo-3 may represent a newly recognized virus. We thus designated the virus tentatively as sambar herpesvirus 1 (SamHV-1 acronym). The other nine sequences shared 97.0% to 100% similarity with that of Elk-LHV (Fig. 1). Although we have tentatively named these samples Elk-LHV, the primary reservoir of this virus remains ambiguous. As far as we know, Elk-LHV has been identified in at least four deer species including elk, red deer, sambar, and milu. Due to the long-term co-evolution process, the host range of a specific γ-HV is moderately narrow [9,15]. However, the broad host spectrum of the Elk-LHV among deer species suggests that the virus might have evolved in an across species manner, a so-called 'species jump' [4].

Phylogenetic assessment based on 31 fragments of γ-HVs DPOL (59 aa) indicated that the viruses were segmented into six clades: *Lymphocryptovirus*, *Macavirus*, *Rhadinovirus* (BoHV-4 like), and three unresolved multifurcated clades, tentatively termed caribou-LHV, Bovid-LHV, and Cervid-LHV (Fig. 2). Members of the *Macaviruses* such as AlHV-1, OvHV-2, MCFV-WTD, etc., are all MCF associated, whereas deer-hosted viruses are mostly clustered within clade caribou-LHV and Cervid-LHV. Viruses identified in this study, together with most deer RuRVs scattered exclusively in the Cervid-LHV with high confidence of an approximate likelihood ratio test (aLRT) support. Notably, the newly identified SamHV-1 had a close relationship with fallow deer-LHV, while the other nine sequences were clustered with Elk-LHV in a distinct branch within this clade, in accordance with the sequence alignment results. The bovid-LHV clade includes viruses from other ruminants such as bovines, sheep, and oryx. The caribou-LHV includes only two closely related viruses, reindeer-LHV, and porcupine-caribou-LHV (Fig. 2).

Previous reports on the evolution of herpesvirus and rhadinoviruses suggested that RuRVs co-evolved with their corresponding reservoirs [5,8,9,15]. However, with its similar DPOL sequences, the phylogenetic tree of this study failed to match that of deer mitochondrial cytochrome b (data not shown). Mismatches in tree topologies were mainly observed in the reindeer-LHV, porcupine-caribou-LHV and mule-LHV taxa in the viral tree, suggesting that these viruses might undergo the species jump phenomenon or undergo independent evolutionary process; otherwise, these deer species are not part of the original reservoir of the viruses.

The present study provides evidence that Sambars could be infected with at least two distinct γ-HVs: one is the newly identified SamHV-1 and the other, an Elk-LHV more ubiquitous among deer species. Like other non-MCF RuRV, neither of those γ-HVs is associated with any clinical indication until now; unfortunately, whether there are more susceptible species remains unknown. Regardless, as a precaution, different deer species should be raised separately as an MCF-like disease occurred in sika deer co-raised with fallow deer according to a retrospective questionnaire from a veterinarian in Inner Mongolia.

Acknowledgments

Conflict of Interest

The authors declare no conflicts of interest.

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