The first study on the occurrence of bovine herpesviruses in the wild fauna of the Moscow region, Russia

Svetlana P. Yatsentyuk, Alexander V. Pchelnikov, Elizaveta R. Safina, and Maria S. Krasnikova

Department of Biotechnology, Russian State Center for Animal Feed and Drug Standardization and Quality, Zvenigorodskoe Highway, Moscow, Russia.

Co-authors: AVP: vetdr-mom@list.ru, ERS: liza-safina@yandex.ru, MSK: m.krasnikova@vgnki.ru

Materials and Methods: One hundred and one tissue samples and nasal swabs of 24 moose and seven roe deer were studied using a real-time polymerase chain reaction (PCR) for BoHV-1 DNA and conventional PCR for BoHV-4 and BoHV-6 DNA. A virus neutralization test (VNT) was used to detect antibodies to BoHV-1 in 19 serum samples. The final antibody titer was calculated with the Spearman-Kärber method.

Results: BoHV-4 and BoHV-6 DNA were not detected in all studied samples of 31 animals. BoHV-1 DNA was detected using a real-time PCR in nasal swabs from 2 adult roe deer. For BoHV-1, only 9/19 tested serum samples reacted positive in VNT with the titer range from 0.67 ± 0.19 to 3.75 ± 0.10 log2. Antibodies were detected in all age groups, more often in fawns under 1-year-old. The seropositivity of females was higher than in males.

Conclusion: Wild ungulates can potentially represent a reservoir of new pathogenic livestock viruses. To study the prevalence and genetic diversity of wild ungulate herpesviruses, detailed molecular studies of the cervid herpesvirus 1, cervid herpesvirus 2, and elk herpesvirus 1 are necessary.

Keywords: antibody, bovine herpesvirus-1, bovine herpesviruses, moose, polymerase chain reaction, roe deer.

Introduction

For a long time, the scientific community was interested in cattle diseases caused by viral pathogens in relation to agricultural production. However, in the past 15 years, there has been an increased interest in studying the spread of such pathogens in populations of wild artiodactyls. Researchers are trying to assess what role wild ungulates play in harboring and transmitting the infection. As more information becomes available, more species of wild animals are being identified as reservoirs of viral pathogens, expanding the range of identified hosts.

It has been shown that different viruses originally found in cattle, can be detected in different species of wild ungulates in the vast areas not only in North America, but also in Europe and Asia [1–4]. Researchers primarily study the seroprevalence of various wild ungulates to herpesviruses, which have a direct economic impact on cattle and sheep livestock [5–8]. The causative agent of infectious bovine rhinotracheitis is bovine alphaherpesvirus 1 (BoHV-1), one of the dangerous pathogens affecting cattle. It belongs to Alphaherpesvirinae subfamily. Primary infection signs might be accompanied by various clinical manifestations, such as rhinotracheitis or vulvovaginitis; the virus can induce abortions in cows, balanoposthitis in bulls, and systemic infections in calves during their 1st months of life. The latest published scientific data confirm the presence of antibodies to BoHV-1 in various groups of wild artiodactyls. According to American researchers, in 2007, the seropositivity of Alaskan caribou (Rangifer tarandus) to BoHV-1 was 47% [9]. Studies conducted in 2011–2012 on yaks (Bos mutus) from different parts of the Qinghai-Tibet Plateau in China showed the presence of antibodies to BoHV-1 in 27.9–44.6% of animals [3]. In 2014, BoHV-1 was isolated from 5 out of 225 buffaloes (Bubalus bubalis) in northeastern Argentina [10]. Studies of 64 wild ruminants from 13 different regions in Iran, including 25 mouflons (Ovis orientalis), 22 wild goats (Capra aegagrus), 9 Indian gazelles (Gazella bennettii), and eight goitered gazelles (Gazella subgutturosa), showed the absence of BoHV-1 antibodies. In contrast, the genetic material of BoHV-1 virus was detected in 1.5% of all cases [8]. In Iran, BoHV-1 strains were

Copyright: Yatsentyuk, et al. Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
isolated from nasal and vaginal swabs in 3 out of 16 buffalo (*B. bubalis*) [11]. A large-scale study of wild ungulates was carried out by various scientific groups in Europe. A study conducted in Poland [12] showed that more than 50% of the studied 1194 wild deer were seropositive to alphaherpesviruses. Antibodies were detected in red deer (*Cervus elaphus*) (25.6%), fallow deer (*Dama dama*) (23.1%), and roe deer (*Capreolus capreolus*) (1.7%). BoHV-1 DNA and antibodies have been reported in populations of Norwegian reindeer (*R. tarandus*) and red deer in Ireland, France, and Belgium [7, 13, 14]. Bovine herpesvirus type 5 (BoHV-5), which also belongs to *Alphaherpesvirinae* [15, 16], circulates in populations of domestic ungulates. Although the presence of the virus in Eastern Europe and the USA cannot be ruled out, BoHV-5 in cattle is more frequently detected in South America (Brazil and Argentina) [16]. At the same time, there is practically no data on the presence of the pathogen in wild animals. Molecular studies show that several viruses that are phylogenetically related to alphaherpesviruses are found in wild ungulates: cervid herpesvirus 1 (CvHV-1), which causes conjunctivitis in red deer, cervid herpesvirus 2 (CvHV-2), and elk herpesvirus 1 (ElkHV-1), which causes subclinical genital infections in reindeer (*R. tarandus*) and elk (*Cervus elaphus canadensis*), respectively. Interestingly, a serological cross-link was established between these viruses and BoHV-1 using ELISA [17, 18]. Representatives of the subfamily *Gammaherpesvirinae*, bovine herpesvirus type 4 (BoHV-4) and herpesvirus type 6 (BoHV-6), have been studied rarely. Since it was discovered in Hungary in 1963, BoHV-4 has been identified in cattle all over the world [19–22]. It was also shown that BoHV-4 can infect American bison (*Bison bison*), buffalos (*Syncerus caffer*), sheep (*Ovis aries*) and goats (*Capra hircus*) [20]. BoHV-6, also known as bovine lymphotropic herpesvirus (BLHV), was first identified in the United States in 1998 in lymphoma samples and bovine peripheral blood cells. It has been later found in cattle in Canada, South America, and New Zealand [23–25]. In 2021, Rosato et al. [26] showed that cattle animals from Switzerland, United Kingdom, Finland, Belgium, and Germany were carriers of BoHV-6 with an overall frequency of 32%, ranging from 22% to 42% in different countries. Based on the data obtained, the authors concluded that BoHV-6 is ubiquitous in healthy cattle. In 2013, BoHV-6 DNA was first detected in nasal swabs of a calf with clinical signs of respiratory pathology in the Moscow region [27]. Furthermore, molecular studies have shown a wide prevalence of BoHV-6 in the same area [28]. Other *Gammaherpesvirinae*, such as elk gammaherpesvirus (Elk-LHV) or fallow deer lymphotropic herpesvirus (LHV), has also been identified in wild ungulates. However, their association with the disease is still unknown. It is believed that they can affect animal’s immune system and cause lymphoproliferative diseases in other species [29, 30]. There is no information on the circulation of these viruses in wild cervids on the territory of Eastern Europe. In 2009, Australian scientists attempted to compare the BoHV-1 seroprevalence in wild animals with the intensity of livestock production in the same area [31]. Their results showed that BoHV-1 was present in wild ruminant populations all around the world, but whether wild ungulates can transmit the viral infection to livestock remained unresolved. There are currently no reports on the prevalence of BoHV-1 in the wild fauna of the Russian Federation.

The research aimed to study the occurrence of BoHV-1 among wild artiodactyls from different areas of the Moscow region in the Russian Federation.

**Materials and Methods**

**Ethical approval**

Ethical approval is not required for this type of study. This study used samples obtained from game animals.

**Study period and location**

The study was conducted from November 2019 to May 2022. The samples were collected from eight districts of the Moscow region (Russia).

**Sample collection**

Samples used in the study were obtained from wild free-living artiodactyls, which were shot during the winter hunting season in 2019 in the Moscow region. Materials were collected from 31 animals, including 24 moose (Alces alces) and seven roe deer (Figure-1).

Animal descriptions and the place of their shooting are presented in Table-1.

For the polymerase chain reaction (PCR) testing, 101 animal tissue samples, including parts of the nasal septum, upper tracheal rings, lung, heart, liver, kidneys, testicles, and nasal swabs, were examined. After the collection, all samples were frozen at the temperature of −18°C before they were studied. For the serological studies, postmortem blood samples were taken from 19 animals by puncture of heart cavities. To obtain serum, tubes with blood were left at room temperature (23°C) for 30 min and then centrifuged at 800× g for 10 min.

**Serological methods**

A virus neutralization test was conducted on serum samples of moose and roe deer with a constant amount of the virus BoHV-1 isolate Kuibyshev-2006(2lTCD₅₀) from the Collection of Strain from the «Federal Scientific Center – All-Russian Research Institute of Experimental Veterinary Medicine – Russian Academy of Sciences», Moscow. Each serum sample was examined in six repetitions.

MDBK cell culture was used for the production of the virus. Cultivation was carried out in polystyrene mats with a growth area of 75 cm² under a non-ventilated lid and at the temperature of 37°C. The growth
nutrient medium, IglaMEM (PanEco, Russia) was supplemented with 7% bovine blood serum. Reseeding of the culture was carried out once a week in a ratio of 1:3. Infection of the cell culture was carried out by the conventional method after the formation of a complete monolayer. Cytopathic effect was monitored daily using a low magnification inverted microscope until a physical detachment of most of the monolayer from the substrate was noticed. Calculation of the infectious titer of the virus was carried out according to the Reed and Muench method [32].

The virus neutralization reaction was carried out using a micromethod in 96-well culture plates with a constant dose of the virus (2lg TCD\textsubscript{50}) according to the generally accepted method. The results were recorded 72 h after the reaction was set up. The final antibody titer was calculated by the Spearman-Kärber method with a 95% confidence interval [33].

DNA extraction and PCR

Tissue samples were homogenized in 10 mL 0.9% sodium chloride. The homogenate was centrifuged at 500× g for 5 min; the supernatant was taken and used for further studies. DNA was extracted from 100 µL of the suspension using the RIBO-prep kit (AmpliSens, Russia). The samples were tested for the presence of BoHV-4 DNA by conventional PCR with specific primers [34].

BoHV-6 DNA conventional PCR reaction was made with specially designed primers. First, all the BoHV-6 sequences were downloaded from the GenBank database (The National Center for Biotechnology Information [NCBI]). Then, the sequences were aligned using the Clustal W algorithm, integrated into the AlignX module of the VectorNTI Advanced 11.0 package (InforMax, Inc., USA). Selected primers flanked the DNA polymerase gene (Dpol) region of BoHV-6 isolate Pennsylvania.

Table 1: Animal descriptions.

| Sample ID | Animal | Sex | Age | Shooting locations |
|-----------|--------|-----|-----|-------------------|
| 1M        | Moose  | F   | >2  | Kolomna district  |
| 2M        | Moose  | M   | >2  | Kolomna district  |
| 3M        | Moose  | F   | >2  | Orekhovo-Zuevo district |
| 4M        | Moose  | F   | >2  | Shatura district  |
| 5M        | Moose  | F   | 5   | Lotoshino district |
| 6M        | Moose  | F   | 3   | Schyolkovo district |
| 7M        | Moose  | M   | 1.5 | Schyolkovo district |
| 8M        | Moose  | F   | >2  | Yegoryevsk district |
| 9M        | Moose  | F   | >2  | Yegoryevsk district |
| 10D       | Roe deer| F   | >2  | Lukhovitsy district |
| 11D       | Roe deer| F   | >2  | Lukhovitsy district |
| 12D       | Roe deer| F   | >2  | Lukhovitsy district |
| 13M       | Moose  | M   | <1  | Pavlovsky-Posad district |
| 14M       | Moose  | F   | 4.5 | Pavlovsky-Posad district |
| 15M       | Moose  | M   | 3   | Shatura district  |
| 16M       | Moose  | F   | 2.5 | Yegoryevsk district |
| 17M       | Moose  | M   | 1.5 | Yegoryevsk district |
| 18M       | Moose  | M   | 1.5 | Shatura district  |
| 19M       | Moose  | M   | <1  | Orekhovo-Zuevo district |
| 20M       | Moose  | F   | >2  | Schyolkovo district |
| 21M       | Moose  | M   | 3.5 | Orekhovo-Zuevo district |
| 22M       | Moose  | F   | <1  | Orekhovo-Zuevo district |
| 23M       | Moose  | M   | 1.5 | Lukhovitsy district |
| 24D       | Roe deer| M   | 2   | Lukhovitsy district |
| 25M       | Moose  | M   | 1.5 | Lukhovitsy district |
| 26D       | Roe deer| F   | >2  | Lukhovitsy district |
| 27D       | Roe deer| F   | >2  | Lukhovitsy district |
| 28D       | Roe deer| F   | >2  | Lukhovitsy district |
| 29M       | Moose  | F   | >2  | Lukhovitsy district |
| 30M       | Moose  | M   | 2   | Lukhovitsy district |
| 31M       | Moose  | F   | <1  | Shatura district  |

*F=Female, M=Male

Figure 1: Localization of animal samples in the Moscow region [Source: Map was constructed using QGIS-OSGeo-4W 3.18.2 program (https://qgis.org/ru/site/index.html)].

Veterinary World, EISSN: 2231-0916
47 (NCBI Reference Sequence: NC_024303.1), which is about 550 bp long. The specificity of primers was studied using the Nucleotide BLAST.

Experimental confirmation of the specificity of the BoHV-6 primers was obtained using DNA of 32 strains of various microorganisms, herpesviruses, and bovine samples. PCR products of expected length from bovine semen and serum samples were studied and confirmed by Sanger sequencing. Afterward, positive samples were used as positive PCR controls.

Amplification was performed in a 25 µL reaction mixture containing 2.5× PCR-mix2 blue (AmpliSens), 10 mM of dNTPs, 0.6 µM of both forward and reverse primers (Table-2) [34]. The conventional PCR reaction was carried out on “Tercky” Multi-block Thermocycler (DNA-technology, Russia) under the conditions illustrated in Table-1. The PCR amplicons were analyzed by running 10 µL of the PCR products on a 1.8% agarose gel stained with ethidium bromide (0.5 µg/mL) in comparison with a GeneRuler 100 bp DNA Ladder (Thermo Scientific™, Lithuania), visualized under the UV light, and photographed by Infinity 1500/36M Xpress Gel documentation system (Vilber Lourmat, France).

Detection of BoHV-1 DNA was based on the amplification of the gE gene fragment using the RINOKOR RT-PCR kit (AmpliSens) on a RotorGene Q (Qiagen, Germany).

Results

Sampling and animal distribution

Animals, 24 moose and seven roe deer, were hunted in 8 districts of the Moscow region. More female (n = 19) than male (n = 12) samples were studied. Animals were classified into three age categories based on their morphological characteristics, including body size, tooth wear, and antler growth: fawns (<1-year-old), yearlings (1–<2-years-old), and adults (≥2-years-old). Most of the animals were adults (n = 22), and 1-year-olds (n = 5) and fawns (n = 4) were approximately equal in quantity.

Serological studies

The results of serological studies are presented in Table-3. Serum samples of 9/31 animals contained antibodies to the BoHV-1 virus with a titer from 0.67 to 3.75. Antibodies were found in serum samples of 3/5 roe deer from the Lukhovitsy district. Out of the 14 tested serum samples taken from moose, BoHV-1 antibodies were detected in six samples from 4 districts of the Moscow region. The maximum titer was detected in the serum sample of an adult moose shot in the Shatura district.

PCR screening

DNA material of BoHV-4 and BoHV-6 was not found by conventional PCR in all 31 animals. At the same time, BoHV-1 DNA was detected using a real-time PCR in samples taken from 2 adult roe deer shot in the Lukhovitsy district of the Moscow region (sample ID 12D - Ct 31.04 and sample ID 27D - Ct 31.54).

Discussion

Samples taken from one of the roe deer contained both BoHV-1 antibodies and viral DNA, which may indicate a prolonged animal contact with the virus. At the same time, in another roe deer BoHV-1 DNA was detected in a nasal swab sample and there were no antibodies in the serum, which may indicate the initial stage of infection. The presence of the BoHV-1 DNA calls attention on the ongoing infection in wild ruminants in the European part of Russia. This does not exclude the possibility that wild ruminants can serve as a reservoir of an infectious agent and, therefore, pose a threat of introducing infection onto the territory of livestock farms.

Since vaccination of wild artiodactyl animals against BoHV-1 is not carried out in Russia, positive results of serological studies may refer to the contact of these animals with the BoHV-1 virus.

All 19 samples for serological studies were collected from districts of the Eastern and Southeastern regions of Moscow (Figure-1). Antibodies to BoHV-1 were discovered in half of these samples (Figure-2). It is impossible to exclude viral circulation in other areas of the Moscow region, where a relatively high density of wild ruminants is observed.

Figure-3 shows the results of seroprevalence tests in different groups. Antibodies to BoHV-1 were found in 60% of the tested roe deer and approximately 43% of the tested moose serum samples. Regarding age-related seroprevalence, it can be concluded that most of the positive serum samples were detected in fawns, then in adult animals, and the least amount was found in 1-year-old animals. Differences in seropositivity of various groups of animals can be explained by the social structure of cervid groups during wintertime. In winter, roe deer form family groups of 10–15 individuals that consist of females with fawns.

**Table-2:** List of oligonucleotides and PCR conditions for BoHV-4 and BoHV-6.

| Virus | Target region | Primer name | Sequence 5’-3’ | Size (bp) | PCR condition | References |
|-------|---------------|-------------|----------------|----------|---------------|------------|
| BoHV-4 | tk | BoHV-4 F BHV-4 R | TTGATAGTCGTTGTTGGATGG CACTGCCCGGTGGGAAATAGCA | 260 | 95°C - 5 min, 45 cycles (95°C - 10 s, 65°C - 20 s, 72°C - 20 s) | [34] |
| BoHV-6 | Dpol | BoHV-6-pol-F BoHV-6-pol-R | ACAGACGGCGACGAGATAAG ATGTTGCCCTGCTAGAGT | 554 | 95°C - 5 min, 42 cycles (95°C - 10 s, 55°C - 10 s, 72°C - 20 s) | This study |

PCR=Polymerase chain reaction, BoHV-4=Bovine herpesvirus type 4, BoHV-6=Bovine herpesvirus type 6
In moose, social structure is similar, but the groups are comprised of 3–4 individuals. In such communities, the transmission of the virus may occur more frequently between females. Yearlings join such groups less often, and fawns, born in summer may still have colostral antibodies. In our study, the seropositivity of the tested female serum samples is higher than those of males, which is also explained by the peculiarity of animal behavior – adult males often live separately.

Our results generally correlate with the data on BoHV-1 seropositivity in wild artiodactyls in other countries. Thus, in yaks living in the Tibetan Plateau in China, level of BoHV-1 antibodies varied from 27.9% to 44.6%, depending on the animal habitat [3]. In the study by Lillehaug et al. [13], more than 3000 Norway cervids serum samples were tested. Antibodies to BoHV-1 were detected in 3% of roe deer samples, but all moose samples were negative. Overall, the value of seropositivity was 28.5%. In a large-scale study of alphaherpesviruses (BoHV-1 and CvHV-1) in free-living ruminants in Poland, seroprevalence ranged from 0% to 100% in different areas [12]. Due to the small number of analyzed samples, our results do not allow us to determine the general seropositivity.
of Moscow region cervids to alphaherpesviruses. However, it is now known that wild artiodactyls that live near Moscow had contact with BoHV-1, and this statement has a correlation with the global trend.

In addition, our study has confirmed the results of the study by Shuliak et al. [35], who demonstrated the presence of infectious bovine rhinotracheitis virus type 1 circulation in wild artiodactyls in Russia. Data from European scientists also validated the fact that BoHV-1 herpesvirus spread among wild artiodactyls in the countries of Eastern Europe, especially Poland, where the virus was noted in fallow deer, red deer, and, to a lesser extent, roe deer [12]. However, in our study, cross-serological reactions with related BoHV-1 herpesviruses in deer (CvHV-1, CvHV-2, and ElkHV-1) cannot be ruled out. For the evaluation of the circulation of these viruses additional PCR studies are required.

**Conclusion**

PCR and serological methods showed that the studied moose and roe deer were in contact with Alphaherpesvirinae BoHV-1, but Gammaherpesvirinae BoHV-4 and DNA were not detected in any of the samples. Further studies on the occurrence of other wild deer herpesviruses are needed to exclude cross-serological reactions with BoHV-1, as well as the studies of a larger number of animals from other districts of the Moscow region.

**Authors’ Contributions**

SY and AVP: Designed the study and drafted the manuscript. AVP: Collected samples. SY and MSK: Conducted PCR testing. AVP and ERS: Performed the serological work. SY: Revised the manuscript. All authors have read and approved the final manuscript.

**Acknowledgments**

This work was supported by the Russian Science Foundation under grant № 22-26-00093 (https://rscf.ru/en/project/22-26-00093/).

**Competing Interests**

The authors declare that they have no competing interests.

**Publisher’s Note**

Veterinary World remains neutral with regard to jurisdictional claims in published map and institutional affiliation.

**References**

1. Casaubon, J., Vogt, H.R., Stalder, H., Hug, C. and Ryser-Degiorgis, M.P. (2012) Bovine viral diarrhea virus in free-ranging wild ruminants in Switzerland: Low prevalence of infection despite regular interactions with domestic livestock. *BMC Vet. Res.*, 8: 204.
2. Conner, M.M., Ebinger, M.R., Blanchong, J.A. and Cross, P.C. (2008) Infectious disease in cervids of North America: Data, models, and management challenges. *Ann. N. Y. Acad. Sci.*, 1134: 146–172.
3. Han, Z., Gao, J., Li, K., Shahzad, M., Nabi, F., Zhang, D., Li, J. and Liu, Z. (2016) Prevalence of circulating antibodies to bovine herpesvirus 1 in Yaks (*Bos grunniens*) on the Qinghai-Tibetan Plateau, China. *J. Wildl. Dis.*, 52(1): 164–167.
4. Kalman, D. and Egyed, L. (2005) PCR detection of bovine herpesviruses from nonbovine ruminants in Hungary. *J. Wildl. Dis.*, 41(3): 482–488.
5. Fabisiak, M., Salamaszynska, A. and Stadejek, T. (2018) Detection of seroconversion to bovine herpesvirus 1 related alphaherpesvirus and bovine viral diarrhea virusin Polish free-living deer. *Pol. J. Vet. Sci.*, 21(3): 437–440.
6. Frolich, K., Hamblin, C., Parida S., Tuppurainen, E. and Schettler, E. (2006) Serological survey for potential disease agents of free-ranging cervids in six selected national parks from Germany. *J. Wildl. Dis.*, 42(4): 836–843.
7. Graham, D.A., Gallagher, C., Carden, R.F.; Lozano, J.M., Moriarty, J. and O’Neill, R. (2017) A survey of free-ranging deer in Ireland for serological evidence of exposure to bovine viral diarrhoea virus, bovine herpes virus-1, bluetongue virus and Schmallenberg virus. *Irel. Vet. J.*, 70: 13.
8. Hemmatzadeh, F., Boardman, W., Alinejad, A., Hematzade, A. and Moghadam, M.K. (2016) Molecular and serological survey of selected viruses in free-ranging wild ruminants in Iran. *PloS One*, 11(12): e0168756.
9. Evans, A.L., das Neves, C.G., Finstad, G.F, Beckmen, K.B, Skjerve, E., Nymo, I.H. and Tryland, M. (2012) Evidence
Available at www.veterinaryworld.org/Vol.15/August-2022/19.pdf

of alphaherpesvirus infections in Alaskan caribou and reindeer. BMC Vet. Res., 8: 5.

10. Maidana, S.S., Konrad, J.L., Craig, M.I., Zabal, O., Mauroy, A., Thiry, E., Crudeli, G. and Romera, S.A. (2014) First report of isolation and molecular characterization of bubaline herpesvirus 1 (BuHV1) from Argentinian water buffaloes. *Arch. Virol.*, 159(11): 2917–2923.

11. Hedaya, N., Hajikolaei, M.R.H. and Seyfi Abad Shapouri, M.R. (2020) Isolation and identification of bubaline herpesvirus 1 (BuHV-1) from latently infected water buffalo (*Bubalus bubalis*) from Iran. *Trop. Anim. Health Prod.*, 52(1): 217–226.

12. Rola, J., Larska, M., Socha, W., Rola, J.G., Materniai, M., Urban-Chmiel, R., Thiry, E. and Zmudzinska, J.F. (2017) Seroprevalence of bovine herpesvirus 1 related alphaherpesvirus infections in free-living and captive cervids in Poland. *Vet. Microbiol.*, 204: 77–83.

13. Lillehaug, A., Vikoren, T., Larsen, I.L., Akerstedt, J., Tharaldsen, J. and Handeland, K. (2003) Antibodies to ruminant alpha-herpesviruses and pestiviruses in Norwegian cervids. *J. Wildl. Dis.*, 39(4): 779–786.

14. Thiry, E., Vercouer, M., Dubuisson, J., Barrat, J., Sepulchre, C., Gerardy, C., Meersschaert, C., Collin, B., Blancou, J., Postepet, P.P. (1988) Serological survey of herpesvirus infections in wild ruminants of France and Belgium. *J. Wildl. Dis.*, 24(2): 268–73.

15. Bartha, A., Hajdu, G., Aldasy, P. and Paczolay, G. (1969) Occurrence of encephalitis caused by infectious bovine rhinotracheitis virus in calves in Hungary. *Acta Vet. Acad. Sci. Hung.*, 19(2): 145–151.

16. Del Medico Zajac, M.P., Ladelfa, M.F., Kotsias, F., Muytkens, B., Thiry, J., Thiry, E. and Romera, S.A. (2010) Biology of bovine herpesvirus 5. *Vir. J.*, 184(2): 138–145.

17. Thiry, J., Widen, F. and Grégoire, F. (2007) Isolation and characterisation of a ruminant alphaherpesvirus closely related to bovine herpesvirus 1 in a free-ranging red deer. *BMC Vet. Res.*, 3: 26.

18. Azab, W., Dayaram, A., Greenwood, A.D. and Osterrieder, N. (2018) How host specific are herpesviruses? Lessons from herpesviruses infecting wild and endangered mammals *Annu. Rev. Virol.*, 5(1): 53–68.

19. Cobb, S.P., Banks, M., Russell, C. and Thorne, M. (2006) Bovine lymphotropic herpesvirus in a UK dairy herd. *Vet. Rec.*, 158(23): 807–808.

20. Mishchenko, V.A., Mishchenko, A.V., Dumova, V.V., Shevchenko, A.A. and Chernyk, O.Y. (2013) *Vet. Kubany*, 2: 11–13.

21. Nefedchenko, A.V., Koteneva, S.V., Glotov, T.I., Glotov, A.G., Yuzhakov, A.G. and Zaberezhnyi, A.D. (2019) Detection of bovine herpesvirus 4 DNA in cattle by real-time PCR. *Vopr Viralol.*, 64(4): 178–184.

22. Williams, L.B.A., Fry, L.M., Herndon, D.R., Franceschi, V., Schneider, D.A., Donofrio, G. and Knowles, D.P. (2019) A recombinant bovine herpesvirus 4 vectored vaccine delivered via intranasal nebulization elicits viral neutralizing antibody titers in cattle. *PLoS One*, 14(4): e0215605.

23. Gagnon, C.A., Allam, O., Drolet, R. and Tremblay, D. (2010) Quebec: Detection of bovine lymphotropic herpesvirus DNA in tissues of an aborted bovine fetus. *Can. Vet. J.*, 51(9): 1021–1022.

24. de Oliveira, C.H., de Oliveira, F.G., Gasparini, M.R., Galinani, G.C., Lima, G.K., Fonseca, A.A. Jr., Barbosa, J.D., Barbosa-Stancioli, E.F., Leite, R.C., Dos Reis, J.K. (2015) Bovine herpesvirus 6 in buffaloes (*Bubalus bubalis*) from the Amazon region, Brazil. *Trop Anim Health Prod.*, 47(2): 465–468.

25. de Boer, M.W., Zheng, T., Baddon, B.M., McDougall, S. (2014) Detection of bovine herpesvirus type 4 antibodies and bovine lymphotropic herpesvirus in New Zealand dairy cows. *N Z Vet. J.*, 62(6):351-355.

26. Rosato, G., Subira, A.R., Al-Saadi, M., Michalopoulou, E., Verin, R., Dettwiler, M., Nordgren, H., Chiers, K., Grobmann, E., Kohler, K., Suntz, M., Stewart, J.P. and Kipar, A. (2021) Gammaherpesvirus infections in cattle in Europe. *Viruses*, 13(12): 2337.

27. Yurov, K.P., Alekseenkova, S.V. and Pchelnikov, A.V. (2021) Detection of Bovine Lymphotropic Gammaherpesvirus in the Nasal Secretions of Sick Calves. In: Theory and Practice of Actual Research: Proceedings of the 5th International Scientific and Practical Conference, Krasnodar, September 17, 2013. Scientific Publishing Center Apriori, Krasnodar. p198–200.

28. Pchelnikov, A.V. and Yatsenyuk, S.P. (2021) Detection of the genetic material of gammaherpesviruses in livestock farms of the Moscow and Tver regions. *J. Vet.*, 7: 22–26.

29. Auer, A., Schweitzer, L., Kühber-Heiss, A., Posauntz, A., Dimmel, K., Seitz, K., Beiglböck, C., Riedel, C. and Rümernapf, T. (2022) Porcine circoviruses and herpesviruses are prevalent in an Austrian game population. *Pathogens*, 11(3): 305.

30. Zhu, H., Liu, H., Yu, X., Zhang, J., Jiang, L., Chen, G., Feng, Z., Li, Y., Feng. T. and Zhang, X. (2018) Evidence of two genetically different lymphotropic herpesviruses present among red deer, sambar, and nilu herds in China. *J. Vet. Sci.*, 19(5): 716–720.

31. Cripps, J.K., Piacconi, C., Scruggie, M.P., Woolnough, A.P. and Ramsey, D.S.L. (2019) Introduced deer and their potential role in disease transmission to livestock in Australia. *Mamm. Rev.*, 49(1): 60–77.

32. Reed, L.J., Muench, H. (1938) A simple method of estimating fifty percent endpoints. *Am. J. Epidemiol.*, 27 (3): 493–497.

33. Thrusfield, M. (1986) Serological epidemiology. In: Thrusfield, M., editor. Veterinary Epidemiology. Butterworths, London, United Kingdom. p175–186.

34. Bayoumi, Y., Sobhy, N., Morsi, A., El-Neshwey, W., El-Seddawy, N. and Abdallah, A. (2021) Clinical and histopathological studies on neurodegeneration and dysautonomia in buffalo calves during foot-and-mouth disease outbreaks in Egypt. *Vet. World*, 14(6): 1622–1630.

35. Shuliak, A.F., Gorbatov, A.V., Stafford, V.V., Loschinin, M.N., Velichko, G.N., Sokolova, N.A., Zhuravleva, E.A., Ishkova, T.A. and Nosova, M.V. (2020) Mixed infection among wild ruminants on hunting grounds. *J. Vet.*, 10: 20–25.

************