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PCR DETECTION OF BOVINE HERPESVIRUSES FROM NONBOVINE RUMINANTS IN HUNGARY

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ABSTRACT: Polymerase chain reaction (PCR) was used to test six different nonbovine ruminant species for five bovine herpesviruses including infectious bovine rhinotracheitis virus (BoHV-1), bovine herpes mammillitis virus (BoHV-2), Movar-type herpesvirus (BoHV-4), bovine herpesvirus type 5 (BoHV-5), and alcelaphine herpesvirus 1 (AlHV-1). Species tested included 56 roe deer (*Capreolus capreolus*), 66 red deer (*Cervus elaphus*), 20 fallow deer (*Dama dama*), 16 mouflon (*Ovis musimon*), 34 domestic sheep, and 44 domestic goats, which were sampled in Hungary in 2003. Tracheal and popliteal lymph nodes collected from these animals were tested for the presence of the five bovine herpesviruses using three nested (two of which were duplex) PCR assays. Three bovine herpesviruses (BoHV-1, -2, and -4) were detected, whereas no evidence of AlHV-1 or BoHV-5 was observed. Prevalence of BoHV-1 ranged from 12% to 47%, and PCR-positive results were observed in all species tested. BoHV-2 was detected from roe deer, red deer, fallow deer, mouflon, and domestic sheep, and the prevalence in these species ranged from 3% to 50%. BoHV-4 was detected in all species, with prevalence ranging from 12% to 69%. Sequenced PCR products were 99-100% identical to bovine herpesviral sequences deposited in the GenBank.

Key words: Alcelaphine herpesvirus 1, AlHV-1, BoHV-1, BoHV-2, BoHV-4, BoHV-5, bovine herpesviruses, cervids, deer, PCR.

INTRODUCTION

Although evidence of bovine herpesviruses in free-ranging wildlife species has been reported, the risk of intraspecies transmission, especially between wildlife and domestic livestock, is poorly understood. Antibodies to bovine rhinotracheitis virus (bovine herpesvirus type 1, BoHV-1) have been reported from seven European countries (Frölich et al., 2002) but less than 1% of red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) proved to be positive for BoHV-1 in France and Belgium (Thiry et al., 1988). BoHV-1 was isolated from water buffalo (*Bubalus carabanesis*) in Malaysia (Ibrahim et al., 1983) and detected by serology in Brazil (Lage et al., 1996). Antibodies to BoHV-1 also have been reported from wildebeest (*Connochaetes taurinus*) and cape buffalo (*Syncerus caffer*) (Rweyemamu, 1970), hippopotamus (*Hippopotamus amphibious*) (Kaminjolo et al., 1970) and black-faced impala (*Aepyceros melampus petersi*) (Karesh et al., 1997) in Africa, and from American bison (*Bison bison*) (Taylor et al., 1997) in the USA. However, these serologic investigations did not differentiate between BoHV-1 and possible cross-reactions with other related herpesviruses.

Alcelaphine herpesvirus 1 (AlHV-1, strain WC-11), which causes wildebeest-associated malignant catarrhal fever (WA-MCF), originally was isolated from blue wildebeest (*Connochaetes taurinus taurinus*) (Plowright et al., 1960). This virus was has been reported from white-bearded (*Connochaetes taurinus albojubatus*) and white-tailed wildebeest (*Connochaetes gnou*) (Seal et al., 1989). Several other herpesviruses have been isolated from African exotic ruminants, deer, bison, gaur (*Bos gaurus*), greater kudu (*Tragelaphus strepsiceros*), and others, but the relation of these viruses to AlHV-1 strain WC-11 has not been reported (Castro et al., 1982; Seal et al., 1989; Blake et al., 1990; Li et al., 2000).

Evidence of malignant catarrhal fever (MCF) infection in free-ranging European ruminants is restricted to a single MCF case that was diagnosed by histopathology in wild moose (*Alces alces*) in Sweden (Warsame and Steen, 1989) and to reports

of seropositive free-ranging fallow deer (*Dama dama*) (Frölich et al., 1998). It is likely that most MCF reports in European deer are associated with sheep-associated MCF (SA-MCF) caused by Ovine herpesvirus 2 (OvHV-2). Evidence of bovine herpesvirus type 2 (BoHV-2) in wild ungulates in Europe is restricted to the detection of antibodies in two female European bison (*Bison bonasus*) that had virus neutralization titers of 20 against BoHV-2 (Borchers et al., 2002).

In contrast to other bovine herpesviruses, bovine herpesvirus 4 (BoHV-4) infects a wide variety of species and replicates in various cell lines. A herpesvirus isolated from thyroid and adrenal glands and from the spleen of an American bison affected with MCF (Todd and Storz, 1983) was identified as BoHV-4 by indirect immunofluorescence assay (IIF) and restriction enzyme cleavage (RE) (Storz et al., 1984). Eleven isolates of BoHV-4 from peripheral blood leukocytes of 45 healthy, one severely ill, and one dead African cape buffalo also were identified as BoHV-4 by IIF and RE (Rossiter et al., 1989). A herpesvirus isolated from a kidney cell culture of a healthy owl monkey (*Aotus trivirgatus*) and another isolate from a cat suffering from urolithiasis later were identified as BoHV-4 strains (Bublot et al., 1991; Fabricant and Gillespie, 1974). This virus also has been isolated from the spleen of a captive lion (*Panthera leo*) (Bartha, pers. comm.; Bartha et al., 1989).

Bovine herpesvirus 5 (BoHV-5) has been associated with rare cases of encephalitis in cattle and was previously considered an encephalitic form of BoHV-1/IBR. Sheep are susceptible to experimental infection (Belák, et al., 1999), but there is no evidence of BoHV-1 infection in European wildlife.

Serological testing for antibodies to bovine herpesviruses can be complicated by cross-reactions between antigenically related viruses and the failure to detect latently infected individuals. In this study, we used PCR to test for four bovine herpesviruses and AlHV-1 in four wildlife species (roe deer, red deer, fallow deer, and mouflon) and two domestic species (goats and sheep) in Hungary.

MATERIALS AND METHODS

Tracheal and popliteal lymph nodes were collected from 66 red deer, 20 fallow deer, 56 roe deer, and 16 mouflon. All samples were from healthy free-ranging animals that were shot by professional hunters from September to December 2003 in all counties of Hungary. Samples were frozen at −20 C for 1–2 mo before PCR testing.

Samples of 34 sheep and 44 goats (lymph nodes and spleens) were also collected from healthy animals that were euthanized at abattoirs and from animals submitted for diagnostic examination to the Veterinary Diagnostic Institute Budapest. All sheep and goat samples originated from northern Hungary.

For sample preparation, thawed tissues were homogenized in mortars and digested with Proteinase-K (Sigma-Aldrich Co., St. Louis, Missouri, USA) at 50 C overnight. Cell debris was sedimented by centrifugation (15,200 × G for 1 min). DNA was purified from supernatant using a Miniprep Express Matrix Kit (Qbiogene Inc., Carlsbad, California, USA) as directed by the manufacturer.

For the detection of BoHV-1 and BoHV-5, a duplex nested PCR with GC1, CR1, and CR26 primers was used (Ros et al., 1999). For the detection of BoHV-4 and AlHV-1, a duplex nested PCR assay was used (Fábián and Egyed, 2004). To detect BoHV-2, a novel nested PCR was developed to amplify a sequence of the glycoprotein H (gH) gene. The gH sequence was obtained from the GenBank European Molecular Biology Laboratory (EMBL) data bank (accession number AF 375976), and the following oligonucleotides were selected as primers: MAM-1: 5′-GTT TGA CGC TGG CTT AGT GG-3′; MAM-2: 5′-TAT CAG GAT TAC CCC GAC CC-3′; MAM-3: 5′-TGA CGC TGG CTT AGT GGG TA-3′, and MAM-4: 5′-CGG TAG GTA TAG ACG GTC GCT C-3′. The outer primers (MAM-1 and MAM-2), flanked a 276-bp fragment; the inner primers (MAM-3 and MAM-4) amplified a 237-bp long product. The PCR amplifications were carried out in 50-μl reaction mixtures containing 5-μl of 10× PCR buffer (100 mM of Tris [pH 9.0], 500 mM of KCl, 1 mg of bovine serum albumin per ml), 100 M of (each) deoxynucleoside triphosphate (Pharmacia Biotech, Uppsala, Sweden), 15 pmol of each primer, 1 U of *Taq* DNA polymerase (Fermentas AB, Vilnius, Lithuania.), 6 μl of 25 mM MgCl2, and 5 μl of sample (max-

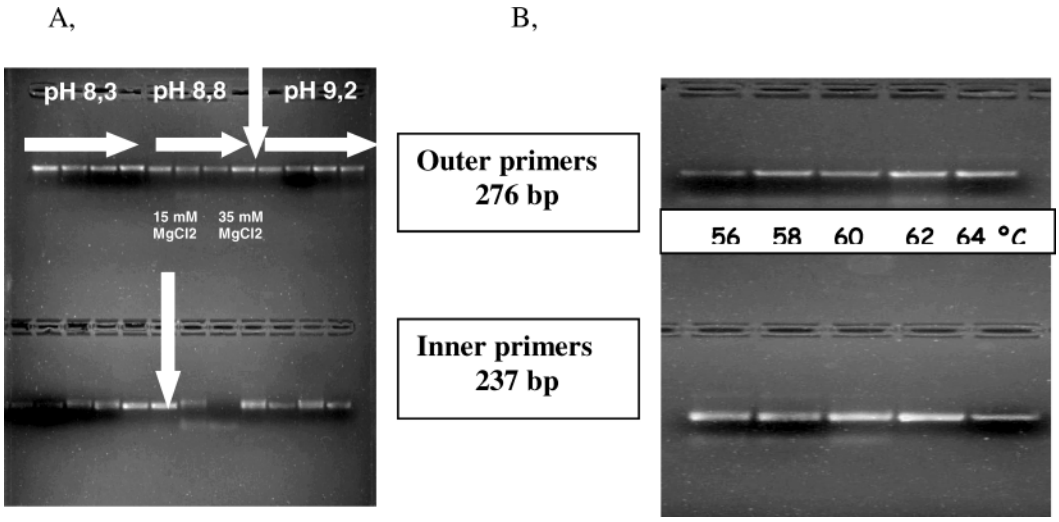

FIGURE 1. Optimizing results for bovine herpesvirus type 2 polymerase chain reaction (PCR): (A) Optimizing kit ($MgCl_2$, KCl, pH) results; (B) Optimal annealing temperatures (Tgradient PCR).

imum of 0.9 μg of total DNA per reaction). The aqueous phase was overlaid with 2–3 drops of mineral oil (Sigma-Aldrich). The 30 amplification cycles included denaturation at 94 C for 45 sec, annealing at 62 C for 1 min, and synthesis at 72 C for 1 min. After the last cycle, the tubes were kept at 72 C for 10 min to complete the extension, and mixtures were cooled to 4 C. From the first PCR product, 1 μl was transferred into the second reaction. Except for modifications in the concentration of $MgCl_2$ (3 μl of 25 mM) and the annealing temperature (64 C), the previously described PCR protocol was followed. For the second round, 62–64 C annealing temperatures seemed to be optimal; we selected the higher temperature to increase specificity. DNA extracted from purified BoHV-2 and distilled water served as positive and negative controls. The optimal annealing temperature (62 and 64 C) was determined by gradient PCR in Tgradient Whatman Biometra device (Analytik GmbH, Goettingen, Germany). For further optimization, the PCR assay was analyzed by a PCR Optimization Kit II (Sigma-Aldrich).

The PCR products were analyzed by electrophoresis in 2% agarose gels using 0.5× Tris-borate-ethylenediaminetetraacetic acid (EDTA) running buffer. Ethidium bromide-stained bands were visualized with ultraviolet (UV) light and recorded with a video camera (Kodak EDAS 290, Hemel, Hempstead, UK). The molecular sizes of fragments were compared with those of a GeneRuler™ 100-bp ladder (Fermentas AB, Vilnius, Lithuania).

Specificity of the assay was checked against 11 animal and human herpesviruses (BoHV-1, -2 [Allerton strain] -4, -5, AlHV-1, equid herpesvirus 1, -2, -5, suid herpesvirus 1, murid herpesvirus 1, human herpesvirus 4). Bovine cell genome was also tested using the Madin-Darby bovine kidney cell line. Sensitivity studies were carried out by determining PCR detection limits from various dilutions of titrated BoHV-2 Allerton strain (100, 10, and 1 plaque forming units [pfu] per reaction). All PCR assays were carried out using previously reported precautions to prevent cross-contamination (Belák and Ballagi-Pordány, 1993). To confirm the specificity of results, one positive field sample, representing each detected virus, was selected for sequencing. The sequences were aligned with the corresponding viral sequences of the data bank by the program BLASTN 2.2.7 (Altschul et al., 1997).

RESULTS

The outer and inner primers used in the BoHV-2 PCR assay could detect 100 pfu in a single reaction (two positives from three reactions). In the nested assay, sensitivity was increased to 1 pfu (five positives from five nested reactions) of BoHV-2. Positive results were not detected for any of the other ten herpesviruses used to assure assay specificity. Amplification with the outer primers was most efficient at higher (30-35 mM) concentration of $MgCl_2$ at pH 8.3 and 9.2 (Fig. 1). The in-

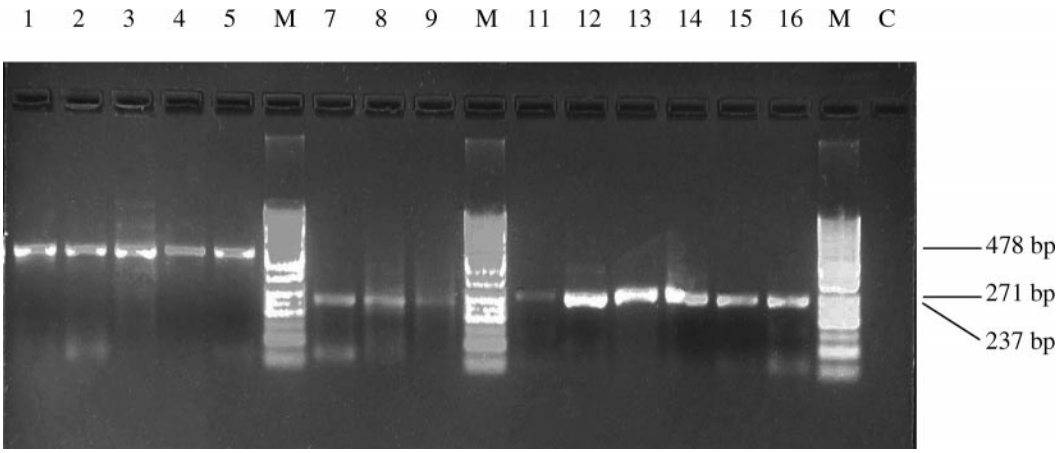

Figure 2. Polymerase chain reaction (PCR) amplification of bovine herpesviruses from nonbovine species. Lines 1–5 BoHV-1 (478 bp), lines 7–9 BoHV-2 (237 bp), lines 11–16 BoHV-4 (271 bp), 100 bp ladder: M, a negative BoHV-2 sample: C. Roe deer: 1, 11. Red deer: 2, 7, 12. Fallow deer: 3, 8, 13. Mouflon: 9. Sheep: 4, 15. Goat: 5, 16.

ner primers were optimized at pH 8.8 at 15 mM of $MgCl_2$. Temperature-gradient PCR indicated peak amplification at 62 C with the outer primers and 64 C with the inner primers.

Positive PCR results were observed for BoHV-1, -2, and -4 but not AlHV-1 or BoHV-5 (Fig. 2). Results for individual species and viruses are shown in Table 1.

Double infections were rare: two roe deer, two red deer, two sheep, and one goat were infected with both BoHV-1 and BoHV-4; one roe deer was infected with BoHV-1 and BoHV-2.

Sequenced PCR products were similar or nearly identical (99-100%) to GenBank sequences of glycoprotein H gene of BoHV-2 (AF375976), glycoprotein C gene of BoHV-1 (AJ004801), and the major capsid protein gene of BoHV-4 (AF318573) and proved to be specific amplifications of bovine herpesviruses. No variant strains, mutations, deletions, or insertions were found.

DISCUSSION

In this study, nested PCR assays were used to detect bovine herpesvirus DNA from lymphoid tissues from nonbovine ruminants. Popliteal lymph nodes were the only available tissue from all tested animals, and although this is not a tissue associated with alpha herpesvirus latency, positive results were detected for BoHV-1, -2, and -4. Samples were restricted to Hungary, and sample sizes for individual species never exceeded 66. For these reasons, prevalence estimates should be approached with some caution, and should not be extrapolated to other wild deer or

TABLE 1. Polymerase chain reaction (PCR) detection of the bovine herpesviruses (BoHV) types -1, -2, -4, and -5 and alcelaphine herpesvirus 1 (AIHV-1) from nonbovine ruminant species.

	BoHV-1	BoHV-2	AIHV-1	BoHV-4	BoHV-5
Roe deer	56/12 (21.4%)	56/4 (7.1%)	56/0	56/7 (12.5%)	56/0
Red deer	66/19 (28.8%)	66/3 (4.5%)	66/0	66/14 (21.2%)	66/0
Fallow deer	20/7 (35.0%)	20/1 (5.0%)	20/0	20/8 (40.0%)	20/0
Mouflon	16/2 (12.5%)	16/8 (50%)	16/0	16/11 (68/7%)	16/0
Sheep	34/16 (47.0%)	34/1 (2.9%)	34/0	34/9 (26.5%)	34/0
Goat	44/13 (29.5%)	44/0	44/0	44/6 (13.6%)	44/0

mouflon populations in Europe. Our data indicate that roe deer, red deer, fallow deer, and mouflon can be infected with bovine herpesviruses and that PCR provides a reliable approach to survey other European populations of these species. Variation in the proportion of infected animals appears to exist between species (Table 1), but additional samples and a more-controlled sampling protocol to address variables that could potentially affect prevalence (e.g., age, location, population density) are needed to fully evaluate these potential differences.

The detection of BoHV-1 in nonbovine species may be most important, especially in relation to the eradication or control of this virus in European livestock. Our results show that the virus is widespread in free-ranging and livestock ruminant species. High numbers of roe deer and red deer exist in Europe, and based on our results, it is possible that a high proportion are infected with BoHV-1. However, the potential for transmission of this virus between species is not fully understood.

Herpes mammillitis exists in the Hungarian cattle population, but clinical signs are only occasionally observed (Rusvai, pers. comm.). The 4–7% prevalence of BoHV-2 among free-ranging deer suggests a low prevalence of infection in these species but provides incomplete information on which to evaluate the reservoir status. Very low infection rates in sheep (3%) and goats (0%) were unexpected and suggest that these domestic species probably do not play a major role in the epidemiology of BoHV-2. The optimized BoHV-2 PCR assay proved to be a suitable tool for detecting BoHV-2 DNA, and it is not believed that these low prevalence estimates reflect problems with assay sensitivity.

Alcelaphine herpesvirus 1 was not detected, even though PCR positives were recently detected in 40% of a Hungarian cattle population (Fábián and Egyed, 2004). However, it is possible that a specificity problem (between AlHV-1 and OvHV-2) may have accounted for the high number of cattle testing PCR-positive. Malignant catarrhal fever in deer and exotic ruminants is frequently reported outside of Europe (Blake et al., 1990; Li et al., 2000; Imai et al., 2001). European red deer are susceptible to experimental infection with MCF (Oliver et al., 1983), and AlHV-1 specific sequences have been amplified from experimentally infected red deer by PCR (Tham et al., 1994).

BoHV-4 infection is well known among various species, especially in ruminants (Goyal and Naeem, 1992; Egyed, 2000). This work indicated widespread infection among all the tested ruminant species, and very similar rates of infection (13–19%) were observed between species.

BoHV-5, as the encephalitic form of IBR, is not a frequent disease in Europe, but there is little survey data available. In Hungary, only one isolation of BoHV-5 exists (Bartha et al., 1969), and only two cases have been recorded (1969 and 1983, Bartha, pers. comm.). For this reason, our negative results were not unexpected.

Sequence analysis of PCR products proved the specificity of the PCR assays; the high identity of DNA sequences to GenBank data did not indicate the presence of closely related herpesviruses or variant strains. However, lack of genetic variation in the relatively short (237–478 bp) PCR products sequenced in this study do not discount these possibilities.

It is disputed whether herpesvirus-infected free-ranging ungulates have direct or indirect epidemiologic connection to livestock. Potential routes for herpesvirus transmission within and between species of wild ruminants and between wild ruminants and domestic livestock are not understood, and in cattle, direct-contact transmission is limited. Our results do not present a clear epidemiologic connection between cattle and free-ranging ruminants. The Hungarian cattle population, in general, is seropositive for BoHV-1, and we found a correspondingly high prevalence (12–35%) of BoHV-1 infected, free-ranging animals, which might suggest

some interspecies connection. In contrast, the two gammaherpesviruses (AlHV-1 and BoHV-4), which infect 40 and 60% of the Hungarian cattle population, respectively (Fábián and Egyed, 2004), were not detected or were detected at a lower rate than reported in cattle. The observed prevalence of bovine herpesviruses in sheep and goats as detected by PCR in this study was very similar to bovine infection rates, which may reflect increased direct contact between sheep, goat, and cattle populations as opposed to free-ranging wildlife species.

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LITERATURE CITED

ALTSCHUL, S. F., T. L. MADDEN, A. A. SCHAFFER, J. ZHANG, Z. ZHANG, W. MILLER, AND D. J. LIPMAN. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Research 2: 3389–3402.

BARTHA, A., G. HAJDÚ, P. ÁLDÁSY, AND G. PACZOLAY. 1969. Occurrence of encephalitis caused by infectious bovine rhinotracheitis virus in calves in Hungary. Acta Veterinaria Hungarica 19: 145–151.

BELÁK, S., AND A. BALLAGI-PORDÁNY. 1993. Experiences on the application of polymerase chain reaction in a diagnostic laboratory. Molecular Cellular Probes 7: 241–248.

———, L. KUCSERA, C. ROS, G. KULCSÁR, L. MAKRANSZKI, T. SOÓS, AND S. BELÁK. 1999. Studies on the pathogenicity of bovine herpesvirus type 5 in sheep. Comparative Immunology Microbiology and Infectious Diseases 22: 207–220.

BLAKE, J. E. N., N. O. NIELSEN, AND W. P. HEUSCHELE. 1990. Lymphoproliferation in captive wild ruminants affected with malignant catarrhal fever: 25 cases (1977–1985). Journal of American Veterinary Medical Association 196: 1141–1143.

BORCHERS, K., J. BRACKMAN, O. WOLF, M. RUDOLPH, M. GLATZEL, M. KRASINSKA, Z. A. KRASINSKI, AND K. FRÖLICH. 2002. Virologic investigations of free-living European bison (Bison bonasus) from the Bialowieza Primeval Forest, Poland. Journal of Wildlife Diseases 38: 533–538.

BUBLOT, M., J. DUBUISSON, M. F. VAN BRESSEM, S. DANYI, P. P. PASTORET, AND E. THIRY. 1991. Antigenic and genomic identity between simian herpesvirus aotus type 2 and bovine herpesvirus type 4. Journal of General Virology 72: 715–719.

CASTRO, A. E., G. G. DALEY, M. A. ZIMMER, D. L. WHITENECK, AND J. JENSEN. 1982. Malignant catarrhal fever in an Indian gaur and greater kudu: Experimental transmission, isolation, and identification of a herpesvirus. American Journal of Veterinary Research 43: 5–11.

EGYED, L. 2000. Bovine herpesvirus type 4: A special herpesvirus (review). Acta Veterinaria Hungarica 48: 501–513.

FÁBIÁN, K., AND, L. EGYED. 2004. Detection of bovine gammaherpesviruses by a nested duplex PCR. Journal of Virological Methods 115: 93–98.

FABRICANT, C. G., AND J. H. GILLESPIE. 1974. Identification and characterization of a second feline herpesvirus. Infection and Immunity 9: 460–466.

FRÖLICH, K., H. LI, AND U. MÜLLER-DOBLIES. 1998. Serosurvey for antibodies to malignant catarrhal fever-associated viruses in free-living and captive cervids in Germany. Journal of Wildlife Diseases 34: 777–782.

———, S. THIEDE, T. KOZIKOWSKI, AND W. JAKOB. 2002. A review of mutual transmission of important infectious diseases between livestock and wildlife in Europe. Annals of the New York Academy of Sciences 969: 4–13.

GOYAL, S. M., AND K. NAEEM. 1992. Bovid herpesvirus-4: A review. Veterinary Bulletin 62: 181–201.

IBRAHIM, A., S. P. SAW, I. FATIMAH, AND A. A. SAHAREE. 1983. Isolation of infectious bovine rhinotracheitis virus from buffalo in Malaysia. Veterinary Record 112: 303–304.

IMAI, K., T. NISHIMORI, R. HORINO, K. KAWASHIMA, H. MURATA, H. TSUNEMITSU, T. SAITO, K. KATSURAGI, AND G. YAEGASHI. 2001. Experimental transmission of sheep-associated malignant catarrhal fever from sheep to Japanese deer (Cervus nippon) and cattle. Veterinary Microbiology 79: 83–90.

KAMINJOLO, J. S., AND J. PAULSEN. 1970. The occurrence of virus-neutralizing antibodies to infectious bovine rhinotracheitis virus in sera from hippopotami and buffaloes. Zentralblatt für Veterinarmedizin 17: 864–868.

KARESH, W. B., A. ROTHSTEIN, W. GREEN, H. O. REUTER, W. E. BRASELTON, A. TORRES, AND A. COOK. 1997. Health evaluation of black-faced impala (Aepyceros melampus petersi) using blood chemistry and serology. Journal of Zoo and Wildlife Medicine 28: 361–367.

LAGE, A. P., R. S. CASTRO, M. I. MELO, P. H. AGUIAR, J. B. BARRETO FILHO, AND L. C. LEITHE. 1996. Prevalence of antibodies to bluetongue, bovine herpesvirus 1 and bovine viral diarrhea/mucosal disease viruses in water buffaloes in Minas Gerais State, Brazil. Revue d'Elevage

et de Medecine Veterinaire de Pays Tropicaux 49: 195–197.

LI, H., N. DRIER, J. KELLER, AND T. B. CRAWFORD. 2000. Newly recognized herpesvirus causing malignant catarrhal fever in white-tailed deer (Odocoileus virginianus). Journal of Clinical Microbiology 38: 1313–1318.

OLIVER, R. E., N. S. BEATSON, A. CATHCART, AND W. S. POOLE. 1983. Experimental transmission of malignant catarrhal fever to red deer (*Cervus elaphus*). New Zealand Veterinary Journal 31: 209–212.

PLOWRIGHT, W., R. D. FERRIS, AND G. R. SCOTT. 1960. Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever. Nature 188: 1167–1169.

ROS, C., M. E. RIQUELME, K. ÖHMAN-FORSLUND, AND S. BELÁK. 1999. Improved detection of five closely related ruminant alphaherpesviruses by specific amplification of viral genomic sequences. Journal of Virological Methods 83: 55–65.

ROSSITER, P. B., I. D. GUMM, D. E. STAGG, P. A. CONRAD, S. MUKOLWE, F. G. DAVIES, AND H. WHITE. 1989. Isolation of bovine herpesvirus-3 from African buffaloes (Syncerus caffer). Research in Veterinary Sciences 46: 337–343.

RWEJEMAMU, M. M. 1970. Probable occurrence of infectious bovine rhinotracheitis virus in Tanzania in wildlife and cattle. Nature 225: 738–739.

SEAL, B. S., R. B. KLIEFORTH, W. H. WELCH, AND W. P. HEUSCHELE. 1989. Alcelaphine herpesviruses 1 and 2 SDS-PAGE analysis of virion polypeptids, restriction endonuclease analysis of genomic DNA and virus replication restriction in different cell types. Archives of Virology 106: 301–320.

STORZ, J., B. EHLER, W. J. TODD, AND H. LUDWIG. 1984. Bovine cytomegaloviruses: Identification and differential properties. Journal of General Virology 65: 697–706.

TAYLOR, S. K., V. M. LANE, D. L. HUNTER, K. G. EYRE, S. KAUFMAN, S. FRYE, AND M. R. JOHNSON. 1997. Serologic survey for infectious pathogens in free-ranging American bison. Journal of Wildlife Diseases 33: 308–311.

THAM, K. M., K. NG, AND L. W. YOUNG. 1994. Polymerase chain reaction amplification of wildebeest-associated and cervine-derived malignant catarrhal fever virus DNA. Archives of Virology 135: 355–364.

THIRY, E., M. VERCOUTER, J. DUBUISSON, A. BARRAT, C. SEPULCHRE, C. GERARDY, C. MEERSSCHAERT, B. COLLIN, J. BLANCOU, AND P. P. PASTORET. 1988. Serological survey of herpesvirus infections in wild ruminants of France and Belgium. Journal of Wildlife Diseases 24: 268–273.

TODD, W. J., AND J. STORZ. 1983. Morphogenesis of a cytomegalovirus from an American bison affected with malignant fever. Journal of General Virology 64: 1025–1030.

WARSAME, I. Y., AND M. STEEN. 1989. Malignant catarrhal fever in wild Swedish moose (Alces alces). Rangifer 9: 51–57.

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