



Short communication

Experimental persistent infection with bovine viral diarrhea virus in white-tailed deer[☆]

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Abstract

Bovine viral diarrhea virus (BVDV) infections cause substantial economic losses to the cattle industries. Persistently infected (PI) cattle are the most important reservoir for BVDV. White-tailed deer (*Odocoileus virginianus*) are the most abundant species of wild ruminants in the United States and contact between cattle and deer is common. If the outcome of fetal infection of white-tailed deer is similar to cattle, PI white-tailed deer may pose a threat to BVDV control programs. The objective of this study was to determine if experimental infection of pregnant white-tailed deer with BVDV would result in the birth of PI offspring. Nine female and one male white-tailed deer were captured and housed at a captive deer isolation facility. After natural mating had occurred, all does were inoculated intranasally at approximately 50 days of pregnancy with 10^6 CCID₅₀ each of a BVDV 1 (BJ) and BVDV 2 (PA131) strain. Although no clinical signs of BVDV infection were observed or abortions detected, only one pregnancy advanced to term. On day 167 post-inoculation, one doe delivered a live fawn and a mummified fetus. The fawn was translocated to an isolation facility to be hand-raised. The fawn was determined to be PI with BVDV 2 by serial virus isolation from serum and white blood cells, immunohistochemistry on skin biopsy, and RT-PCR. This is the first report of persistent infection of white-tailed deer with BVDV. Further research is needed to assess the impact of PI white-tailed deer on BVDV control programs in cattle.

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1. Introduction

Infections with bovine viral diarrhea virus (BVDV) occur globally and are cause for substantial economic and genetic losses to the beef and dairy industries. Two distinct BVDV species exist, BVDV 1 and BVDV 2,

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which are referred to as the genotype. In addition, BVDV occurs in two biotypes, non-cytopathic (ncp) and cytopathic (cp), which is determined by the effects of a strain on cells in culture. Most BVDV infections occurring naturally are caused by ncp strains of BVDV. There are two forms of infection associated with BVDV: acute or transient infection and persistent infection. Acute infections are post-natal infections in an immunocompetent host. In contrast, persistent infection only occurs by *in utero* infection of the developing fetus with an ncp BVDV prior to the development of immunocompetence (Brownlie et al., 1987). The virus is recognized as a self-antigen, and the animal is considered immunotolerant and persistently infected (PI) with BVDV. In the epidemiology of BVDV, PI animals constitute the major source of transmission of the virus within and among cattle herds, and maintain the virus in a population (Wittum et al., 2001). Until recently, reports of persistent infections occurring in species other than cattle have been limited to traditional domestic animals, such as sheep, goats, and swine. Within the past three years, persistent BVDV infections in other heterologous species have been reported. Identification of PI alpacas (*Lama pacos*) and lesser Malayan mousedeer (*Tragulus javanicus*) has indicated there may be reservoirs of BVDV in species other than cattle (Mattson et al., 2006; Uttenthal et al., 2005). Simpson (2002) suggested wildlife reservoirs of BVDV may be responsible for failures of BVDV eradication programs that cannot be traced to introduction of or contact with PI cattle (Simpson, 2002).

The white-tailed deer (*Odocoileus virginianus*) is the most abundant wild ruminant species in many regions of North America. Estimates of total number, while widely variable, suggest there are at least 15 million, but possibly up to 30 million animals in the United States (Curtis and Sullivan, 2001). Considering the abundance of white-tailed deer, contact between cattle and deer is common. Over the last 50 years, a number of virological and serological studies have been conducted to assess BVDV infection of white-tailed deer, and results suggest that this species can be infected and possibly maintain the virus in the population (Chase et al., 2004; Friend and Halterman, 1967; Kahrs et al., 1964; Richards et al., 1956; Sadi et al., 1991; Van Campen et al., 1997). To date, BVDV infection of pregnant white-tailed deer resulting in the birth of a PI fawn has not been reported; however, there have been indications

this may be possible (Chase et al., 2004; Van Campen, 2002). The objective of this study was to determine if experimental infection of pregnant white-tailed deer with two genotypes of BVDV would result in the birth of PI offspring. In cattle, the epidemiological importance of PI animals is well recognized. Identification of persistent BVDV infection in white-tailed deer may be influential when planning for BVDV control or eradication programs.

2. Materials and methods

2.1. Experimental inoculation of deer with BVDV

The work reported herein was performed under the approval of the Institutional Animal Care and Use Committee of Auburn University (2005–0909). In December 2005, nine female and one male white-tailed deer were captured from the wild as previously described (Ditchkoff et al., 2005). All animals were determined to be negative for BVDV and BVDV antibodies on virus isolation and virus neutralization, respectively, at initial capture. Natural mating occurred in captivity with peak breeding season estimated to be centered around January 20, 2006. On March 11, 2006, the nine does were sedated and examined ultrasonographically, either transrectally and/or transabdominally, to determine pregnancy status and gestation length. When possible, the number of fetuses per dam and the fetal crown to rump length were measured to estimate fetal ages, according to previously described methods (Hamilton et al., 1985). All pregnant white-tailed deer were inoculated with the ncp BVDV 1 strain BJ and the ncp BVDV 2 strain PA131. The BVDV strains were propagated in Madin-Darby bovine kidney (MDBK) cells in Eagle's minimum essential medium (EMEM), supplemented with 10% equine serum, L-glutamine, penicillin G (100 units/mL), and streptomycin (100 µg/mL). Virus was harvested from cells by a single freeze-thaw method, titered, and stored (–80 °C) until the day of inoculation. Each doe was inoculated by intranasal administration with 10⁶ cell culture infective doses (50% endpoint) (CCID₅₀) of the BVDV 1 BJ and BVDV 2 PA131 strains diluted in 2 mL of EMEM (1 mL per nostril).

Following inoculation, pregnancies were allowed to advance without interference until parturition,

which was expected to occur from the middle of August until the beginning of September 2006. A fawn was born in August, and was moved to an isolation facility and housed individually while BVDV testing was performed. Blood was collected for virus isolation, virus neutralization and RT-PCR procedures, nasal swabs were collected for virus isolation, and skin was collected for immunohistochemistry (IHC). With exception of IHC on skin biopsies, sample collections and virological procedures were repeated on days 31 and 60 of life.

Approximately 30 days after the last doe had been expected to give birth (206 days post-inoculation), all adult deer were sedated, and blood samples were collected for virus isolation and virus neutralization procedures. Following blood collection, all deer were euthanized using a lethal injection of barbiturate. Gross pathologic examinations were performed, and samples of spleen, mesenteric lymph node and uterus were collected for virus isolation procedures.

2.2. Virus isolation

Virus isolation procedures were performed on serum, whole blood, nasal swabs, and tissues collected at post-mortem examination. Samples were assayed for BVDV by passage through MDBK cells, according to previously described methods (Givens et al., 2003). Following passage, identification of BVDV was performed with an immunoperoxidase monolayer assay using BVDV-specific monoclonal antibodies D89 and 20.10.6 (Givens et al., 2003).

2.3. Skin biopsy immunohistochemistry

Immunohistochemical detection of BVDV antigen was performed on formalin-fixed paraffin-embedded skin biopsies using a monoclonal antibody, 15C5 (Brodersen, 2004). The 15C5 monoclonal antibody reacts with an epitope of the E^{RNS} of BVDV that is shared by diverse BVDV isolates and therefore is a suitable target for the detection of a wide variety of isolates of BVDV, including BJ and PA131.

2.4. Virus neutralization

A standard virus neutralization microtiter assay was used for the detection and quantification of

antibodies in serum (Givens et al., 2003). Sera obtained at initial capture were tested for neutralizing antibodies to the cp BVDV 1 strain NADL and the cp BVDV 2 strain 125C. The sera collected from adult deer at the time of inoculation and post-mortem examination, and from the fawn were tested for neutralizing antibodies to the ncp BVDV 1 BJ and ncp BVDV 2 PA131 strains with which the deer had been experimentally inoculated. MDBK cells were used as the indicator cells. Each test included a back titration of the virus and a positive and negative serum control. Following a 5-day incubation period, antibody titer was defined as the inverse of the highest dilution with complete inhibition of cytopathic effect (sera collected at initial capture) or complete inhibition of staining by the immunoperoxidase test (sera collected from adult deer at inoculation and pathologic examination, and from the fawn).

2.5. Reverse transcriptase polymerase chain reaction and sequencing of virus

The BVDV was detected by a two-round rapid-cycle PCR assay on blood samples obtained from the fawn. This reverse transcription nested PCR (RT-nPCR) has been previously described in detail (Givens et al., 2001). All steps of the RT-nPCR were performed in a single-tube reaction. In the first round, the outer primers, BVD 100 (5'-GGCTAGCCATGCCCTTAG-3') and HCV 368 (5'-CCATGTGCCATGTACAG-3') amplified a 290 base pair sequence of the 5' untranslated region of the viral genome. In the second round of the reaction, the inner primers BVD 180 (5'-CCTGAGTACAGGGDAGTCGTCA-3') and HCV 368 amplified a 213 base pair sequence within the first amplicon. After completion of the PCR cycle, 5 μ L of the RT-nPCR products were separated by 1.5% agarose gel electrophoresis. Ethidium bromide staining allowed visualization of the RT-nPCR product using an ultraviolet transilluminator.

Sequence analysis was performed on aliquots of the RT-nPCR products carried out in triplicate. Samples were purified and sequenced by automated dye terminator nucleotide sequencing using both the 5' and 3' primers (BVD 180 and HCV 368, respectively). Consensus sequences were determined for each sample using Align X[®] computer software (Vector NTI Suite 7.1, InforMax, Inc., Bethesda, MD, USA)

and compared to nucleotide sequences of the challenge strains of BVDV.

3. Results

On the day of inoculation, ultrasound examination revealed that all does were pregnant with 1–2 fetuses (mean: 1.625). Gestational age of fetuses was estimated to be between 30 and 80 days (mean: 52.9). Stress from capture and inoculation caused mortality in five does, which were found on post-inoculation day 1. Necropsies and tissue collections for virological procedures were performed on all does. No gross lesions were noted in the deceased animals. Virus isolation from tissue samples (spleen, lung, liver, kidney, placenta, and embryo/fetus) did not yield positive results.

On August 25, 2006 a male fawn was found and immediately removed from the captive deer pen. The fawn was dry, appeared bright and alert, and was able to ambulate. The fawn was estimated to be approximately 12 h old. The birth weight of the fawn was 1.5 kg (3.3 lbs), which is at the low end of body-weights reported previously for neonatal white-tailed deer in Alabama (Haugen, 1959). A mummified fetus was found in immediate proximity of the fawn, lying in the same birthing area as the fawn. A crown-to-rump length measurement of the mummified fetus indicated that the fetus had died at approximately 94 days of pregnancy (Hamilton et al., 1985), approximately, 50 days after inoculation.

At the time when no further parturitions were expected to occur, all adult deer were sedated and humanely euthanized. All does were determined to be non-pregnant at this time and no further mummified fetuses were found. Serum virus neutralization was positive in all adult animals, including the buck that had not been inoculated (Table 1). Virus was not isolated from serum and tissue samples collected after euthanasia.

After translocation of the fawn to the isolation facility, blood samples and a skin biopsy were taken for virological examinations. Neutralizing antibodies were not detected by standard virus neutralization. Serum and white blood cell fractions tested positive for BVDV on virus isolation. The IHC on the skin biopsy also yielded a positive result, indicated by

Table 1

Serum antibody titers of adult deer on day of inoculation and 206 days thereafter

	BVDV 1 (BJ)		BVDV 2 (PA131)	
	Day 0	Day 206	Day 0	Day 206
Doe 1	<1:2	1:128	1:2	1:1024
Doe 3	<1:2	1:64	<1:2	1:1024
Doe 5	<1:2	1:128	<1:2	1:2048
Doe 6	<1:2	1:64	<1:2	1:512
Buck	<1:2	1:16	<1:2	1:512

uptake of immunoperoxidase stain by skin tissues (Fig. 1). The IHC staining pattern from the fawn resembled skin biopsies obtained from PI calves. Nested RT-PCR was positive on serum and whole blood of the fawn, corroborating the results of virus isolation (Fig. 2). The PCR product of the nested RT-nPCR was submitted for sequencing, and analysis of the sequence revealed BVDV 2. Furthermore, sequencing demonstrated the BVDV isolate from the fawn was the BVDV 2 strain PA131 that had been used at inoculation. With exception of the skin biopsy IHC, all virological examinations were repeated on samples taken from the fawn on days 31 and 60 post-partum. Virus isolation from whole blood, serum and nasal swabs and RT-nPCR tested positive for BVDV validating the PI status of the fawn. The titer of BVDV neutralizing antibodies remained <1:2. At the time of

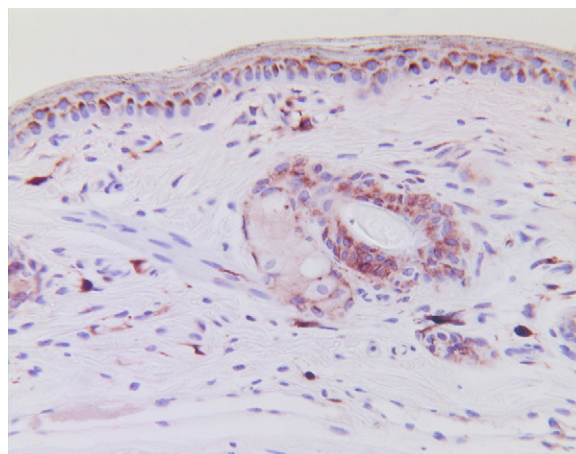


Fig. 1. Positive immunohistochemical staining for BVDV antigen in epidermal and hair follicle epithelium from a fawn persistently infected with BVDV (note reddish-brown staining). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

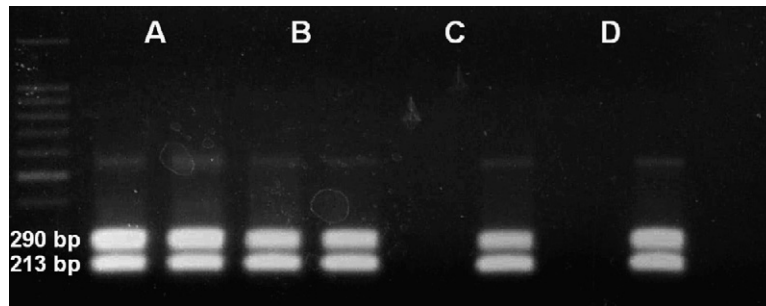


Fig. 2. Agarose gel electrophoresis of the amplification products generated from a persistently infected white-tailed deer using primers specific for bovine viral diarrhea virus. (A) Serum from fawn in duplicate; (B) whole blood from fawn in duplicate; (C) negative and positive control for RNA extraction; (D) negative and positive control for RT-nPCR.

submission of this publication (day 94 of life), the fawn has remained free from clinical signs of disease and has developed normally.

4. Discussion

Our work demonstrates that experimental infection of pregnant white-tailed deer (*O. virginianus*) with BVDV may result in the birth of PI offspring. This is the first published report of experimentally induced persistent BVDV infection in this species; however, previous reports indicate that natural infection with BVDV may cause persistent infection in white-tailed deer (Chase et al., 2004). Interestingly, like the BVDV isolate causing persistent infection in our study, a BVDV isolate obtained from a wild white-tailed deer was also determined to belong to genotype 2 (Chase et al., 2004). These findings contrast recent reports of persistent infections in other heterologous species, such as mousedeer and alpacas, where BVDV 1 was the PI strain (Carman et al., 2005; Grondahl et al., 2003; Mattson et al., 2006). In our study, pregnant does were inoculated with strains of BVDV from both genotypes in order to determine which genotype of BVDV is more suitable to result in PI offspring. The inoculation procedure used in this study was based on a protocol developed for the evaluation of BVDV vaccines in cattle using a multiple BVDV strain challenge model (Brock and Chase, 2000). Unlike in that protocol, where both genotypes of BVDV were isolated from whole blood samples of PI fetuses, only BVDV 2 was isolated from blood samples of the PI fawn. In the study by Brock and Chase (2000), the

BVDV 2 strain was isolated more consistently from tissues samples when compared to BVDV 1, indicating a more global replication of BVDV 2 and possibly a better host adaptation of this strain (Brock and Chase, 2000). This observation may be emphasized by the markedly higher antibody titer against BVDV 2 compared to BVDV 1 that was detected in all adult animals at time of euthanasia. Furthermore, the time of virological examination may have contributed to the observed differences. While samples were examined 60 days post-inoculation in the cattle study (Brock and Chase, 2000), samples from the fawn were examined 167 days post-inoculation. Further research is needed to determine the significance of BVDV genotype in causing the PI state in white-tailed deer.

Persistent infection of white-tailed deer may need consideration, when planning BVDV eradication or control strategies in the United States. White-tailed deer have been recognized as a wildlife reservoir host for different pathogens, including *Ehrlichia chaffeensis* and *Mycobacterium bovis* (Lockhart et al., 1997; Schmitt et al., 2002). The identification of *M. bovis* in white-tailed deer in Michigan is believed to be the result of a spill-over infection from cattle (Schmitt et al., 2002), and this demonstrates how increasing numbers of white-tailed deer in conjunction with changing management factors of wildlife and cattle populations can affect the dynamics of an infectious disease (O'Brien et al., 2002). Infectibility with a pathogen alone does not cause a species to be a wildlife reservoir. Other factors, such as sufficient transmission as a result of consistent shedding of the infectious agent, sufficient contact between species, and maintenance of the infectious agent within a

wildlife population are necessary to constitute a reservoir species. Whether these factors are present to make white-tailed deer a wildlife reservoir for BVDV is not known. The central role of PI cattle in the epidemiology of BVDV and the findings of this study make it plausible that white-tailed deer may constitute a wildlife reservoir for BVDV.

Reproductive deficiency, including reduced conception rates, early embryonic deaths, and abortions are commonly associated with BVDV infection of pregnant animals (Grooms, 2004). The cause of pregnancy loss in most of the experimentally infected does can only be speculated because the use of wild, captive white-tailed deer for this study precluded close observation. Potential causes for fetal loss following capture and inoculation include hypoxemia associated with capture and administration of sedatives, or BVDV infection. Hypoxemia and hyperthermia are common side effects of chemical immobilization of deer (Read et al., 2001).

By our calculations, the dam of the PI fawn and mummified fetus was inoculated on day 43 of gestation. To our knowledge, the fetal age when the development of the immune system in white-tailed deer occurs is unknown; therefore, determining the gestational age at which BVDV may cause persistent infection in white-tailed deer is difficult to predict. Immune system competence of bovine fetuses occurs prior to day 125–150 of gestation. Experimental inoculation of pregnant heifers with the same strains of BVDV 1 and BVDV 2 used in this study on day 75 (± 5) of gestation induced the PI state in all fetuses (Brock and Chase, 2000). Extrapolation of these data was the basis for the mean day of gestation chosen for experimental inoculations of white-tailed deer. The gestation length for white-tailed deer is approximately 200–205 days. The gestational timeframe at which persistent infection in white-tailed deer can occur is likely to be similar to cattle, where fetuses in the first and early second trimester are most susceptible to becoming PI. Further research is needed to determine the gestational age when PI infection in fetal white-tailed deer may occur.

References

- Brock, K.V., Chase, C.C.L., 2000. Development of a fetal challenge method for the evaluation of bovine viral diarrhoea virus vaccines. *Vet. Microbiol.* 77, 209–214.
- Brodersen, B.W., 2004. Immunohistochemistry used as a screening method for persistent bovine viral diarrhoea virus infection. *Vet. Clin. North. Am. Food Anim. Pract.* 20, 85–93.
- Brownlie, J., Clarke, M.C., Howard, C.J., Pocock, D.H., 1987. Pathogenesis and epidemiology of bovine virus diarrhoea virus infection of cattle. *Ann. Rech. Vet.* 18, 157–166.
- Carman, S., Carr, N., DeLay, J., Mohit, B., Dereg, D., Hazlett, M., 2005. Bovine viral diarrhoea virus in alpaca: abortion and persistent infection. *J. Vet. Diagn. Invest.* 17, 589–593.
- Chase, C.C.L., Braun Pearce, L.J., Leslie-Steen, P., Graham, T., Miskimmins, D., Ridpath, J., 2004. Isolation of Bovine Viral Diarrhoea Virus from white-tail deer in Southeastern South Dakota. In: *Bovine Viral Diarrhoea Virus in the Americas Symposium*, University of California, Davis.
- Curtis, P.D., Sullivan, K.L., 2001. White-tailed deer. In: *Wildlife Damage Management Fact Sheet Series*, Cornell Cooperative Extension, Ithaca, NY. http://wildlifecontrol.info/ccewdmp/Publications/Deer_factsheet.pdf#search=%22white-tailed%20deer%20quality%20deer%20management%22.
- Ditchkoff, S.S., Raglin, J.B., Smith, J.M., Collier, B.A., 2005. From the field: capture of white-tailed deer fawns using thermal imaging technology. *Wildlife Soc. Bull.* 33, 1164–1168.
- Friend, M., Halterman, L.G., 1967. Serologic survey of two deer herds in New York State. *Bull. Wildlife Dis. Assoc.* 3, 32–34.
- Givens, M.D., Galik, P.K., Riddell, K.P., Stringfellow, D.A., Brock, K.V., Bishop, M.D., Eilertsen, K.J., Loskutoff, N.M., 2001. Validation of a reverse transcription nested polymerase chain reaction (RT-nPCR) to detect bovine viral diarrhoea virus (BVDV) associated with in vitro-derived bovine embryos and co-cultured cells. *Theriogenology* 56, 787–799.
- Givens, M.D., Heath, A.M., Brock, K.V., Brodersen, B.W., Carson, R.L., Stringfellow, D.A., 2003. Detection of bovine viral diarrhoea virus in semen obtained after inoculation of seronegative postpubertal bulls. *Am. J. Vet. Res.* 64, 428–434.
- Grondahl, C., Uttenthal, Houe, H., Rasmussen, T.B., Hoyer, M.J., Larsen, L.E., 2003. Characterisation of a pestivirus isolated from persistently infected mouset Deer (*Tragulus javanicus*). *Arch. Virol.* 148, 1455–1463.
- Grooms, D.L., 2004. Reproductive consequences of infection with bovine viral diarrhoea virus. *Vet. Clin. North Am. Food Anim. Pract.* 20, 5–19.
- Hamilton, R.J., Tobin, M.L., Moore, W.G., 1985. Aging fetal white-tailed deer. In: *Annual Conference of the Southeastern Association of Fish and Wildlife Agencies*. pp. 389–395.
- Haugen, A.O., 1959. Breeding records of captive white-tailed deer in Alabama. *J. Mammal.* 40, 108–113.
- Kahrs, R., Atkinson, G., Baker, J.A., Carmichael, L., Coggins, L., Gillespie, J., Langer, P., Marshall, V., Robson, D., Sheffy, B., 1964. Serological studies on the incidence of Bovine Virus Diarrhoea, infectious Bovine Rhinotracheitis, Bovine Myxovirus Parainfluenza-3, and *Leptospira Pomona* in New York State. *Cornell. Vet.* 54, 360–369.
- Lockhart, J.M., Davidson, W.R., Stallknecht, D.E., Dawson, J.E., Howerth, E.W., 1997. Isolation of *Ehrlichia chaffeensis* from wild white-tailed deer (*Odocoileus virginianus*) confirms their role as natural reservoir hosts. *J. Clin. Microbiol.* 35, 1681–1686.

- Mattson, D., Baker, R., Catania, J., Imbur, S., Wellejus, K., Bell, R., 2006. Persistent infection with bovine viral diarrhoea virus in an alpaca. *J. Am. Vet. Med. Assoc.* 228, 1762–1765.
- O'Brien, D.J., Schmitt, S.M., Fierke, J.S., Hogle, S.A., Winterstein, S.R., Cooley, T.M., Moritz, W.E., Diegel, K.L., Fitzgerald, S.D., Berry, D.E., Kaneene, J.B., 2002. Epidemiology of mycobacterium bovis in free-ranging white-tailed deer, Michigan, USA, 1995–2000. *Prev. Vet. Med.* 54, 47–63.
- Read, M.R., Caulkett, N.A., Symington, A., Shury, T.K., 2001. Treatment of hypoxemia during xylazine-tiletamine-zolazepam immobilization of wapiti. *Can. Vet. J.* 42, 861–864.
- Richards, S.H., Schipper, I.A., Eveleth, D.F., Shumard, R.F., 1956. Mucosal disease of deer. *Vet. Med.* 51, 358–362.
- Sadi, L., Joyal, R., St-Georges, M., Lamontagne, L., 1991. Serologic survey of white-tailed deer on Anticosti Island, Quebec for bovine herpesvirus 1, bovine viral diarrhoea, and parainfluenza 3. *J. Wildlife Dis.* 27, 569–577.
- Schmitt, S.M., O'Brien, D.J., Bruning-Fann, C.S., Fitzgerald, S.D., 2002. Bovine tuberculosis in Michigan wildlife and livestock. *Ann. N.Y. Acad. Sci.* 969, 262–268.
- Simpson, V.R., 2002. Wild animals as reservoirs of infectious diseases in the UK. *Vet. J.* 163, 128–146.
- Utenthal, A., Grondahl, C., Hoyer, M.J., Houe, H., van Maanen, C., Rasmussen, T.B., Larsen, L.E., 2005. Persistent BVDV infection in mousedeer infects calves: do we know the reservoirs for BVDV? *Prev. Vet. Med.* 72, 87–91.
- Van Campen, H., 2002. Bovine viral diarrhoea virus in wildlife. In: *Detecting and Controlling BVDV Infections Conference Proceedings*, Ames, IA, p. 19.
- Van Campen, H., Williams, E.S., Edwards, J., Cook, W., Stout, G., 1997. Experimental infection of deer with bovine viral diarrhoea virus. *J. Wildl. Dis.* 33, 567–573.
- Wittum, T.E., Grotelueschen, D.M., Brock, K.V., Kvasnicka, W.G., Floyd, J.G., Kelling, C.L., Odde, K.G., 2001. Persistent bovine viral diarrhoea virus infection in US beef herds. *Prev. Vet. Med.* 49, 83–94.