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Review

Biology of bovine herpesvirus 5

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ABSTRACT

Bovine herpesvirus 5 (BoHV-5) is an alphaherpesvirus responsible for meningoencephalitis in young cattle and is closely antigenically and genetically related to bovine herpesvirus 1 (BoHV-1). Both viruses have common aspects in their pathogenesis: (1) they infect epithelial cells at the portal of entry and (2) they establish a latent infection in the sensory nerve ganglia, i.e., the trigeminal ganglia. However, they have different neuroinvasion and neurovirulence capacities. Only in rare cases can BoHV-1 reach the brain of infected cattle. BoHV-5 infection induces different degrees of severity of neurological disease depending on both viral and host factors. Although a case of BoHV-5 associated disease in Europe and some outbreaks in USA and Australia have been reported, the current geographical distribution of BoHV-5 infection is mainly restricted to South America, especially Brazil and Argentina. This review focuses on the genomic characteristics, pathobiology and epidemiology of BoHV-5, in order to provide information on the possible basis of alphaherpesvirus neuropathogenesis.

Introduction

Bovine herpesviruses 1 (BoHV-1) and 5 (BoHV-5) are two closely related alphaherpesviruses that infect cattle. The first case of bovine meningoencephalitis associated with a herpesvirus infection was reported in Australia by Johnston et al. (1962). Based on virion morphology, cytopathic effect in cell culture and antigenic properties, the isolated virus was considered to be a neuropathogenic variant of BoHV-1 (French, 1962).

Comparative studies of this agent with different BoHV-1 strains based on restriction site mapping of viral DNA (Brake and Studdert, 1985), cross-neutralisation tests (Bagust and Clark, 1972; Metzler et al., 1986) and monoclonal antibody reactivity (Metzler et al., 1986) indicated that these strains of virus differ in genomic and antigenic properties. Thus, the previously named bovine encephalitis herpesvirus (BEHV) (Studdert, 1989) or BoHV-1 subtype 3 (Metzler et al., 1986) was recognised as a distinct virus species by the International Committee on Taxonomy of Viruses in 1992 (Roizman et al., 1992) and reclassified as BoHV-5.

BoHV-5 infection induces either a subclinical infection or disease of moderate severity in adult cattle (Ashbaugh et al., 1997; Cascio et al., 1999; Del Médico Zajac et al., 2006) and lethal encephalitis in young animals (Bartha et al., 1969; Carrillo et al., 1983a; Meyer et al., 2001). Cases of BoHV-5 associated disease have been reported in the USA (Reed et al., 1973; D'Offay et al., 1993), Australia (French, 1962; Johnston et al., 1962), Brazil (Riet-Correa et al., 1989, 2006; Salvador et al., 1998), Argentina (Carrillo et al., 1983b; Perez et al., 2003) and seldom in Europe (Moretti et al., 1964; Bartha et al., 1969).

Although BoHV-5 and BoHV-1 are genetically and antigenically related, they differ in their neuroinvasion and neurovirulence capacities. BoHV-1 neuroinvasion usually does not go further than the first order neurone located in the trigeminal ganglion, where the latent infection is established, whereas BoHV-5 is able to infect different regions of the brain (Carrillo et al., 1983a; Perez et al., 2002; Vogel et al., 2003). However, a few cases of BoHV-1 associated encephalitis have been reported (Roels et al., 2000; Silva et al., 2007).

The study of BoHV-5 associated disease and infection linked to the characterisation of the virus provide useful information on the genetic basis of alphaherpesvirus neuropathogenesis. Since its differentiation from BoHV-1 in 1992, a considerable amount of data has accumulated on the BoHV-5 genome, pathobiology and epidemiology, as well as on the available diagnostic methods. These aspects are reviewed in the present article.

Aetiological agent

BoHV-5 belongs to the Family Herpesviridae, Subfamily Alphaherpesvirinae, Genus *Varicellovirus*. BoHV-5 strains are classified

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into three subtypes based on viral DNA analysis by restriction endonuclease fingerprinting (Fig. 1) (Metzler et al., 1986; D'Arce et al., 2002). Type strains for subtypes a, b and non-a-non-b, are the Australian strain N569, the Argentinean strain A663 and Brazilian strains, respectively.

The viral structure consists of a core containing linear double-stranded DNA, an icosahedral capsid, an amorphous-appearing material – the tegument – and a phospholipid envelope containing viral glycoprotein spikes on its surface (Roizman and Pellet, 2007) (Fig. 2).

BoHV-1 and BoHV-5 have a 'D' type genome arrangement, consisting of a unique long fragment (UL) and a unique short fragment (US) flanked by repeat sequences (terminal and internal repeats, TR and IR, respectively) (Roizman and Pellet, 2007) (Fig. 3). Virion DNA includes equimolar amounts of DNA molecules differing in the US orientation relative to the UL region and, at least in BoHV-1, low levels (5%) of genomes having the UL segment in an inverted orientation (Schynts et al., 2003). These data obtained for BoHV-1 can reasonably be extrapolated to the BoHV-5 genome.

The BoHV-5 genome is 137,821 base pairs (bp) long and is approximately 2 kb longer than the BoHV-1 genome, with a G + C base composition of 75% (Delhon et al., 2003). The BoHV-5 genome contains 70 genes, all of which are present in BoHV-1; however, BoHV-5 lacks a homologue of UL 0.5 (Delhon et al., 2003). The UL region contains 60 putative genes. The US region contains eight genes and is flanked by the TR and IR elements. TR and IR each contain a copy of two regulatory genes.

BoHV-1 and BoHV-5 exhibit an average of 82% amino acid identity amongst different proteins. The most similar proteins ($\geqslant$95% amino acid identity) are those involved in viral DNA replication and processing of virion proteins (Delhon et al., 2003) (Table 1). The least conserved sequences between both viruses ($\leqslant$75%) are thought to be involved in the control of the virus cycle, in virus-cell

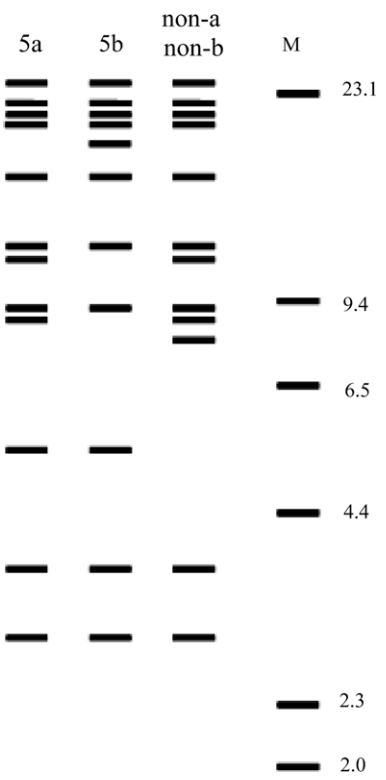

Fig. 1. Scheme of the *Bst*EII restriction pattern of different bovine herpesvirus 5 strains showing subtypes a, b, non-a-non-b. M: Molecular weight marker. Modified figure reproduced from D'Arce et al. (2002) with permission of Elsevier.

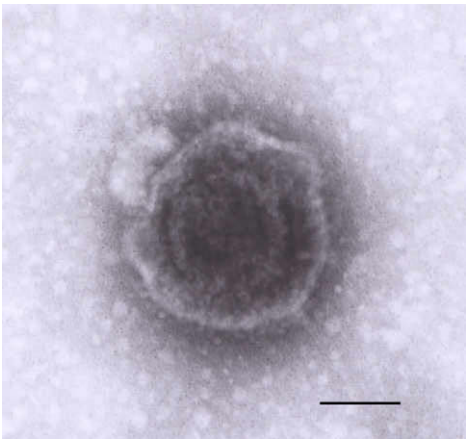

Fig. 2. Herpesvirus morphology as visualised by transmission electron microscopy of an intact negatively stained BoHV-5 virion. The diameter of the virion is approximately 260 nm. The black bar indicates 100 nm.

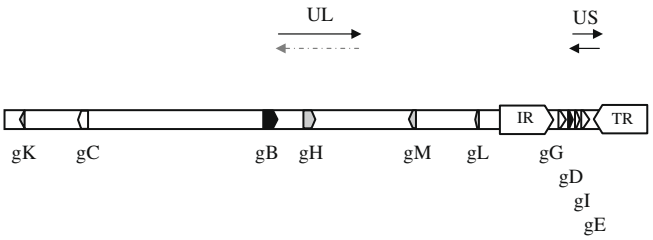

Fig. 3. Schematic representation of BoHV-5 genome. The unique long (UL) and short (US) sequences are shown and their possible orientation is indicated by arrows. The US region is flanked by two repeated and inverted sequences (internal and terminal repeats, IR and TR, respectively). The UL region is 104,054 bp long, the US region is 9548 bp long and the TR and IR elements have 12,109 bp each. Localisation and orientation of the genes encoding glycoproteins are indicated by oriented boxes. The colour code is as follow: black filling indicates that the mean conservation (at the protein level) between BoHV-5 and BoHV-1 gene is higher than 90%; grey filling indicates a conservation level higher than 80%; white filling indicates a conservation level lower than 80%.

Table 1
Genes showing the highest and the lowest amino acid conservation between bovine herpesviruses 1 and 5.

Gene name	% Identity	Gene product and/or function
Most conserved		
UL5	99	Component of the helicase-primase complex
UL48	98	*Trans* inducing factor (α TIF – VP16)
UL39	97	Ribonucleotide reductase (large subunit)
UL19	97	Major capsid protein (VP5)
UL14	97	Minor tegument protein
UL29	95	Single stranded DNA binding protein
UL15	95	DNA cleavage-packaging protein (terminase)
Least conserved		
UL44	75	Glycoprotein C (gC)
UL3	75	Phosphoprotein
BICP4	75	Positive and negative gene regulator
UL24	74	Putative membrane associated protein
US8	74	Glycoprotein E (gE)
UL11	73	Myristylated tegument protein
UL49	72	Tegument protein
US4	72	Glycoprotein G (gG)
BICP0	70	IE transactivator protein
UL3.5	69	Virion protein
LR ORF2	69	Latency related product
US2	69	Tegument protein
BICP22	68	Transcription factor
UL0.7	41	Unknown

interaction and in viral neuropathogenesis (Delhon et al., 2003) (Table 1).

Viral glycoproteins play important roles in virulence by mediating attachment and entry of the virion into the host cell, fusion and cell-to-cell spread (Mettenleiter, 2003). In addition, because of their location in the virion envelope and on the surface of infected cells, glycoproteins are important targets for the host immune response (Schwyzer and Ackermann, 1996). The BoHV-5 genome encodes 10 glycoproteins, namely, gK (UL53), gC (UL44), gB (UL27), gH (UL22), gM (UL10), gL (UL1), gG (US4), gD (US6), gI (US7) and gE (US8) (Delhon et al., 2003). The protein N is encoded by the UL49.5 gene and is considered to be a 'false' glycoprotein in BoHV-1 because it is not glycosylated. However, there are no data concerning this glycoprotein in BoHV-5 (Liang et al., 1996).

BoHV-5 belongs to a group of closely related ruminant viruses where BoHV-1 is the prototype. The genetic relationship between these viruses has been studied firstly based on the glycoprotein B (gB) gene sequence (Ros and Belak, 2002). The percentage of nucleotide identity between BoHV-5 and the other ruminant alphaherpesviruses is 87.4% with BoHV-1, 97.9% with bubaline herpesvirus 1 (BuHV-1), 91.5% with cervid herpesvirus 1 (CvHV-1), 74% with cervid herpesvirus 2 (CvHV-2) and 83.1% with caprine herpesvirus 1 (CpHV-1) (Ros and Belak, 2002). Refined analyses, including UL27 (gB) and US8 (gE) genes sequences, show that BuHV-1 is the most closely related virus to BoHV-5, followed by BoHV-1, CvHV-1, ElkHV-1 and CvHV-2 and, more distantly, CpHV-1 (Ros and Belak, 2002; Thiry et al., 2007b) (Fig. 4).

Pathogenesis

Entry and dissemination

There are scarce data regarding BoHV-5 transmission. Viral DNA and infectious virus have been detected in semen of subclinically infected bulls (Gomes et al., 2003). BoHV-1 is transmitted by direct contact with infected animals (nose to nose contact or genital contact at mating), or by aerosol, and indirect transmission may occur through contaminated food, water or fomites and by semen, including artificial insemination (Muylkens et al., 2007).

BoHV-5 initially infects epithelial cells at the portal of entry, i.e., replication occurs in the nasal or vaginal mucosa, depending on the route of infection (Bagust, 1972; Bagust and Clark, 1972). BoHV-5 viral replication in the respiratory system leads to a high production of progeny virus that is shed in the nasal secretions and constitutes a very efficient source of transmission during acute infection.

Experimental intravaginal, epidural and intracerebral inoculations lead to neurological disease and death (French, 1962; Bagust, 1972; Bagust and Clark, 1972). Animals inoculated by the intraconjunctival route develop hyperthermia, with nasal replication of the virus, but they do not show any nervous signs (Bagust, 1972). After initial replication, spread of virus probably occurs by three routes, as described previously for BoHV-1: local dissemination, systemic spread by viraemia and neuronal spread (Pastoret et al., 1982; Engels and Ackermann, 1996).

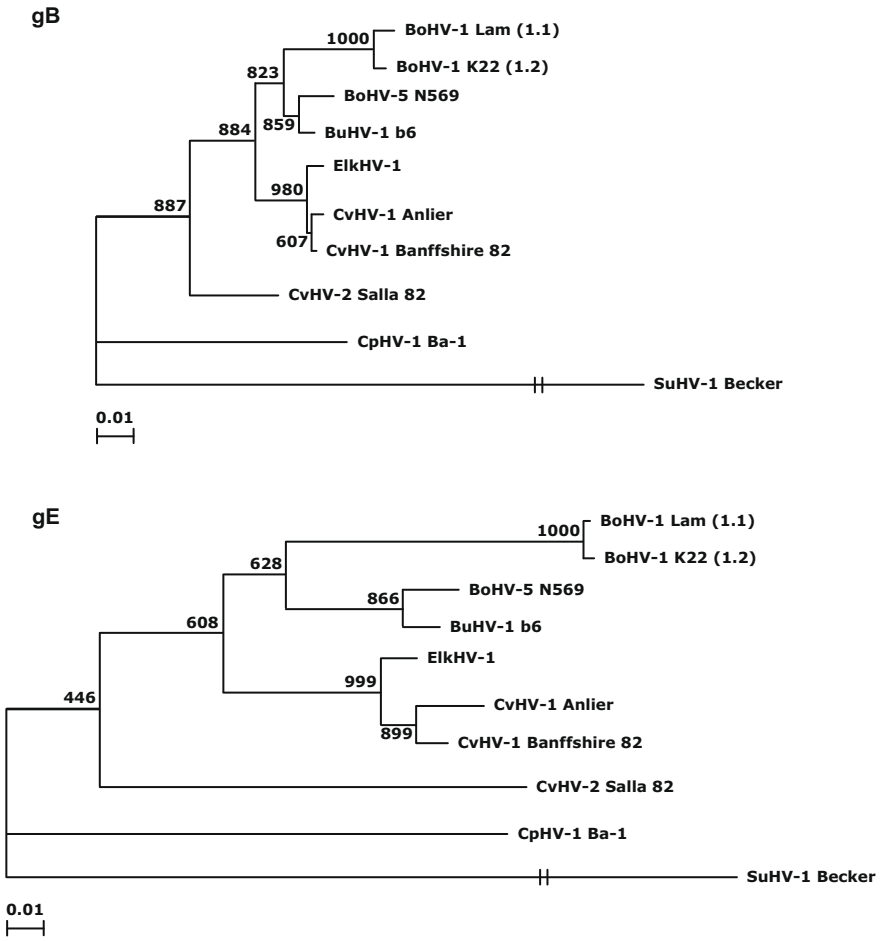

Fig. 4. Phylogenetic relationships between ruminant alphaherpesviruses. Trees were generated by using selected parts of UL27 (a) or US8 (b) nucleotide sequences and were both rooted by using suid herpesvirus (SuHV-1) Becker strain gB sequence as an outgroup. Reproduced from Thiry et al. (2007b) with permission of BioMed Central.

Local dissemination in the infected mucosa could be accounted for by two different mechanisms previously described for BoHV-1: (1) viral particles released in the extra-cellular medium are able to infect susceptible cells; and (2) direct cell-to-cell spread, i.e., viral transmission from an infected cell to nearby uninfected cells.

Systemic spread by viraemia has been indirectly demonstrated by virus isolation from liver, kidney and leucocytes after intranasal and intravenous inoculation (Bagust and Clark, 1972; Belknap et al., 1994; Meyer et al., 2001). However, this route of dissemination does not appear to be significant in BoHV-5 pathogenesis.

After a high rate of primary viral replication in the nasal epithelium, BoHV-5 infects local nerve cells (olfactory cells and/or maxillary nerve endings) and reaches the central nervous system (CNS) by neurone to neurone spread by two different routes: the olfactory and trigeminal pathways. The presence of virus in the trigeminal nerves and microscopic changes in trigeminal ganglia appear at 5–7 days post-infection (dpi) (Bagust, 1972; Bagust and Clark, 1972; Perez et al., 2002). However, the simultaneous development of lesions in the anterior cerebral cortex, medulla, pons and trigeminal ganglia suggests that BoHV-5 also reaches the brain by axonal transport from bipolar neurones of the olfactory nerves to the olfactory bulbs (Perez et al., 2002).

Nevertheless, other routes of brain invasion, such as sympathetic and parasympathetic systems observed in herpes simplex virus (HSV) and pseudorabies virus (PrV) infections (Mettenleiter, 2003), should be investigated.

Once the virus has reached the CNS, it usually induces a fatal meningoencephalitis in cattle after 8–10 dpi (Belknap et al., 1994; Meyer et al., 2001; Perez et al., 2002); however, some cases of subclinical infection (Ashbaugh et al., 1997; Cascio et al., 1999; Vogel et al., 2003) or moderate disease (Del Médico Zajac et al., 2006) have been reported. If animals survive, a lifelong latent infection is established in the sensory ganglia (Cascio et al., 1999; Meyer et al., 2001; Perez et al., 2002) and, under certain conditions, reactivation of the virus and reinfection of the peripheral tissue could occur.

Neurovirulence

Viral neurovirulence is defined as the pathogenicity of a certain virus (or strain) at the CNS level. It has been suggested that neurovirulence could also be due to host factors, such as the genetic background and the immunological status.

Viral factors

The genes and their encoded proteins involved in the neurovirulence of alphaherpesviruses are classified in three groups: enzymes involved in nucleic acid metabolism, factors that modulate the immune response and viral glycoproteins.

Viral polymerase encoded by the UL30 gene has been strongly associated with neurovirulence in equid herpesvirus 1 (EHV-1) (Nugent et al., 2006). Variation of a single amino acid in the polymerase of EHV-1 strains responsible for neurological disease is associated with neurovirulence. This variant amino acid occurs at a highly conserved position for herpesvirus DNA polymerases. The protein encoded by the UL48 gene is a transactivator and its association with neurovirulence for PrV has been assessed in mice (Klopfleisch et al., 2006; Fuchs et al., 2007). Since these two proteins are present in BoHV-5 genome, their role in neurovirulence should be investigated.

Regarding proteins that modulate the immune response, the HSV-1 key neurovirulence determinant ICP34.5 is not present in BoHV-5 (Delhon et al., 2003). On the other hand, HSV-1 ICP47, which interferes with MHC I presentation, has no homologous gene in BoHV-5, but this mechanism is present (Ambagala et al., 2004). Identification of the viral factor is still pending.

Several studies have been undertaken on the characterisation of the role of BoHV-5 glycoproteins in neurovirulence using the rabbit model. Intranasal inoculation of rabbits with a BoHV-5 gC negative virus showed that this virus replicates less efficiently in the brain than the wild type virus, suggesting that gC may regulate BoHV-5 neurotropism in some areas of the rabbit olfactory pathway (Chowdhury et al., 2000a). BoHV-5 gE negative virus had a reduced neural invasion (the virus was found up to the olfactory bulb-second order neurones) and neurovirulence (Chowdhury et al., 2000b). Glycoprotein I seems not to be relevant in neuroinvasion and/or neurovirulence of the virus (Al-Mubarak and Chowdhury, 2004). A BoHV-5 US9 negative virus was almost avirulent in rabbits after intranasal infection (Chowdhury et al., 2002). The mutant virus infects the olfactory receptor neurones (first-order neurones in the olfactory pathway), but it is not transported to the bulb by anterograde transport.

Moreover, calves infected with BoHV-5 gI/gE/US9-deleted virus by the intranasal route showed reduced viral excretion compared with the wild type virus and did not develop nervous signs (Hubner et al., 2005). Virus was recovered only from the basal portions of the CNS (pons and mesencephalum) in very low titres and did not reactivate after dexamethasone treatment. Based on these results, the authors suggested a reduced ability of the recombinant virus to spread from peripheral neurones to CNS neurones in calves. These results support the suggested role of gI, gE and US9 in anterograde transport of the virus in the rabbit model (Chowdhury et al., 2000b, 2002).

Other alphaherpesvirus neurovirulence factors include mostly tegument proteins. For PrV, studies revealed that deletion of UL3.5, UL11, UL16, UL21, UL36 and UL43 genes affected neurovirulence in the mice model. These genes have their homologues in BoHV-5 but their role in neurovirulence remains unknown (Klopfleisch et al., 2006; Bottcher et al., 2008).

Host factors

BoHV-5 infection is usually associated with a lethal neurological disease at the CNS level, although some animals develop a subclinical infection or a moderate disease. These differences in virulence have been associated both with viral and host factors, especially the age and the immunological status. On the other hand, BoHV-1 induces respiratory and/or genital disease and can infect neurones from the peripheral nervous system; however, some cases of encephalitis caused by BoHV-1 infection have been reported in adult cattle (Roels et al., 2000; Silva et al., 2007). These sporadic cases probably reflect individual host susceptibilities to CNS infection rather than BoHV-1 strain modifications, giving rise to an increased neuroinvasion and/or neurovirulence.

The involvement of interferon (IFN) α and β in the pathogenesis of bovine herpesvirus infection is supported by experiments performed in transgenic mice (Abril et al., 2004). Animals lacking the IFN-α/β receptor, which were unable to produce mature B and T lymphocytes (i.e., mice with deletion of both copies of the RAG-2 gene), died after BoHV-5 infection. Mice with RAG-2 gene deletions and lacking both IFN α/β and IFN γ developed BoHV-1 and BoHV-5-associated disease and died after infection (Abril et al., 2004).

Latency and reactivation

The main site of latent infection of alphaherpesviruses after ocular, nasal or oral infection is the sensory nerve ganglia, in par-

ticular the trigeminal ganglia (Rock et al., 1992; Meyer et al., 2001; Perez et al., 2002). After the application of a natural stimulus or treatment with glucocorticoids, reactivation occurs in latently infected animals (Meyer et al., 2001; Perez et al., 2002; Vogel et al., 2003). Viral replication could lead to re-excretion and spread of the virus to susceptible surrounding animals.

In cattle latently infected with BoHV-1, viral DNA was also present in germinal centres of the pharyngeal tonsil (Winkler et al., 2000), peripheral blood cells (Fuchs et al., 1999), lymph nodes and spleen (Mweene et al., 1996).

In latently infected animals, BoHV-5 DNA was detected in several areas of the CNS (mainly olfactory cortex, cerebellum, thalamus, midbrain and pons) (Meyer et al., 2001; Perez et al., 2002; Vogel et al., 2003). The nasal and tracheal mucosae also have been suggested as possible sites of BoHV-5 latency (Meyer et al., 2001). After dexamethasone-induced reactivation of latently infected calves, the distribution of BoHV-5 DNA was altered, affecting the dorsolateral and posterior cortices and, compared to acute infected animals, the anterior cortex, medulla oblongata and cervical medulla were more consistently positive (Vogel et al., 2003).

Histopathological examination of brain sections from reactivated calves suggests that dissemination of BoHV-5 to the cerebral tissue after reactivation would occur via the trigeminal nerve. This result underlines a difference in CNS viral invasion pathways between the acute and latent phases of BoHV-5 infection, since it has been suggested that the virus reaches the brain more directly by the olfactory nerves in acute infection (Perez et al., 2002).

BoHV-5 can be reactivated and re-excreted without clinical signs (Belknap et al., 1994; Cascio et al., 1999) or cattle may develop clinical manifestations of encephalitis similar to those observed during acute infection (Perez et al., 2002; Vogel et al., 2003). The former situation is of particular interest, since animals latently infected would act as natural reservoirs of the virus and represent a silent source of viruses in re-excretion conditions.

Clinical signs

The first signs detected after BoHV-5 infection consist of depression, anorexia and general weakness. Approximately 5 days later, neurological signs, such as incoordination, blindness, muscular tremor, circling, head pressing, recumbency followed by convulsions, paddling and death, are observed (Bartha et al., 1969; Salvador et al., 1998). In some outbreaks, nasal and ocular secretions are observed prior to neurological signs, whilst only a few cases exhibit hyperthermia (Bartha et al., 1969; Carrillo et al., 1983a, 1983b; Schudel et al., 1986). In addition, BoHV-5 virus was isolated from an aborted foetus (Salvador et al., 1998).

There have been several attempts to experimentally reproduce BoHV-5 infection. After intranasal inoculation of the virus, nasal replication and mild respiratory signs, such as nasal and ocular discharges and sneezing, were frequently observed before the onset of neurological signs (Bagust, 1972; Bagust and Clark, 1972; Belknap et al., 1994; Meyer et al., 2001; Perez et al., 2002; Del Médico Zajac et al., 2006). In only a few cases lesions are found in the nasal mucosa (Bagust, 1972).

Neurological signs are initiated from 8–13 dpi, leading to death 2–5 days after the onset of clinical signs. Although BoHV-5-induced encephalitis is usually fatal, some experimentally infected animals develop subclinical infection (Ashbaugh et al., 1997; Cascio et al., 1999; Vogel et al., 2003) or moderate disease, with recovery after 24 dpi (Del Médico Zajac et al., 2006). The different degrees of severity of neurological disease induced by BoHV-5 infection could be due to viral strains factors, as well as age and immunological status of the animals.

Immune response

To date, very few studies have been performed to elucidate the immune response induced after BoHV-5 infection. However, due to the similarities between BoHV-1 and BoHV-5, it is reasonable to conclude that both viruses provoke similar immune mechanisms in the host during infection.

In BoHV-1 infections, the first response of the host is constituted by an inflammatory and innate immune response. The adaptive response starts at 5 dpi, with the induction of specific cells ($CD4^+$ and $CD8^+$ lymphocytes), showing its highest activity at 10 dpi (Denis et al., 1994; Babiuk et al., 1996). The humoral immune response is detected from 10 dpi (Romera et al., 2001). BoHV-1 antibodies participate in the neutralisation of extracellular viral particles and in the induction of antibody-dependent cell mediated cytotoxicity. The antibody response is critical in preventing secondary infections and limiting the consequences of reactivation (Babiuk et al., 1996).

Cattle experimentally infected with BoHV-5 exhibit a lymphoproliferative response at 7 and 14 dpi (Del Médico Zajac, 2007). These animals show total serum antibodies from day 12 dpi and both IgA and IgG_1 isotypes are detected in nasal secretions. Neutralising antibodies are first detectable at 14 dpi (Meyer et al., 2001; Perez et al., 2002; Vogel et al., 2003; Del Médico Zajac, 2007).

Epidemiology

Host range

Cattle are the natural host of BoHV-5. In young animals (up to 6 months of age), the course of the disease is rapid and lethal (Carrillo et al., 1983a; Schudel et al., 1986). Adult animals are considered to be less susceptible to BoHV-5 infection, depending on viral strains and host characteristics. Latently infected animals constitute natural reservoirs of the virus that could potentially contaminate susceptible animals after reactivation.

Natural occurrence of BoHV-5 antibodies has been reported in sheep (Lindner et al., 1993). Experimental infections of sheep and goats showed that both species are susceptible to BoHV-5, developing acute and latent infection (Belak et al., 1999; Silva et al., 1999; Diel et al., 2007). In addition, both species re-excrete virus after dexamethasone treatment and transmission of the virus amongst sheep during acute infection has been detected (Silva et al., 1999). These results suggest that goats and sheep may potentially be infected under natural conditions and act as reservoirs of the virus. Although these studies did not assess the ability of both species to transmit the virus to cattle, the ability to establish and reactivate latent infections could have potential implications in BoHV-5 epidemiology in areas where these species are raised in close contact.

Currently, the animal model available to study BoHV-5 pathogenesis is the rabbit model (Chowdhury et al., 1997). Mice are hitherto assumed to be resistant to infection with either BoHV-5 or BoHV-1, although transgenic mice have been successfully infected with both viruses (Abril et al., 2004).

Geographical distribution

BoHV-5 outbreaks are sporadic and appear to be restricted in their geographical distribution, being mostly detected in the Southern hemisphere. The reasons for this particular distribution are still undetermined. Only a few cases of BoHV-5-associated encephalitis have been detected in Australia (French, 1962; Johnston et al., 1962), North America (Reed et al., 1973; D'Offay et al., 1993) and Europe (Bartha et al., 1969; Moretti et al., 1964). Outbreaks are most commonly reported in Brazil (Riet-Correa

et al., 1989, 2006; Salvador et al., 1998) and Argentina (Carrillo et al., 1983b; Schudel et al., 1986; Perez et al., 2003).

Cross-protection by naturally occurring or vaccine-induced BoHV-1 antibodies has been suggested as a possible explanation for the rare occurrence of BoHV-5-associated disease in BoHV-1 endemic areas, such as Europe and the USA (D'Offay et al., 1993). However, the epidemiological situation in South America does not support this hypothesis. Even though Argentina and Brazil have a high percentage of BoHV-1 seropositive cattle (24.8–84.1% and 19–85%, respectively) (Odeón et al., 2001; Gularte Quincozes, 2005), both countries have reported several cases of BoHV-5 associated meningoencephalitis. The reason for the discrepancy of the prevalence between South America and the rest of the world is unknown. Another important issue is that South American countries do not have any specific programme for the detection and identification of BoHV-5 infected animals. Furthermore, a differential serological test that allows the identification of BoHV-1 and/or BoHV-5 positive animals is not available. Thus, the true prevalence of this infection is unknown and the economic relevance remains to be determined.

Diagnosis

Post mortem examination

The main macroscopic lesions reported in the brain after natural or experimental BoHV-5 infection consist of softening of the parenchymal tissue, focal meningeal haemorrhages in the frontal and/or ventral areas and haemorrhagic foci in the pons and left parietal lobe (Carrillo et al., 1983a; Perez et al., 2002). Gross respiratory lesions, such as nasal congestion, petechial haemorrhages and congestion of the pharyngeal and laryngeal mucosa (Carrillo et al., 1983a), as well as bronchopneumonia (Belknap et al., 1994; Meyer et al., 2001), have also been detected.

Histopathology

Microscopic lesions consist of widespread non-suppurative meningitis characterised by perivascular cuffing, neuronophagia, satellitosis, focal and diffuse gliosis, haemorrhages and neuronal necrosis and degeneration (Bagust and Clark, 1972; Belknap et al., 1994; Meyer et al., 2001; Perez et al., 2002). In some cases, inclusion bodies are observed in astrocytes and neurones (Bagust and Clark, 1972; Carrillo et al., 1983b).

Virological diagnosis

Virological assays are the best available tools to specifically diagnose BoHV-5 infections. The techniques can be performed with nasal secretions, semen or post mortem samples. Virus isolation in cell culture is a suitable assay to be used with fresh or sometimes frozen samples.

In order to differentiate BoHV-5 and BoHV-1, several assays are available: restriction enzyme analysis (REA) (Brake and Studdert, 1985; D'Arce et al., 2002), immunoassays using monoclonal antibodies (Metzler et al., 1986; Keuser et al., 2004; Thiry et al., 2007a), PCR followed by REA (Ros and Belak, 1999), nested PCR (Ashbaugh et al., 1997), multiplex PCR (Alegre et al., 2001; Claus et al., 2005; Thiry et al., 2007b), random amplified polymorphic DNA (RAPD) (Afonso et al., 2007) and, recently, multiple PCR sequencing assays (Del Médico Zajac, 2007).

Serological diagnosis

BoHV-1 and BoHV-5 are antigenically very similar. Several in vitro experiments have shown that both viruses share common epitopes (Metzler et al., 1986; D'Arce et al., 2002). Serum neutralisation assays demonstrate cross-reactions between BoHV-1 and BoHV-5 (Meyer et al., 2001). BoHV-1 antibodies neutralise BoHV-5 virus twofold less than BoHV-5 antibodies and vice versa.

The only currently available assay that is able to differentiate antibodies against BoHV-1 from BoHV-5 is a BoHV-1 gE blocking ELISA, already used to distinguish between BoHV-1 infected or vaccinated animals (Wellenberg et al., 2001). However, this BoHV-1 gE blocking ELISA has not been validated over a large number of samples. Indeed, one might speculate, due to the high similarity between BoHV-5 and BoHV-1, that gE seropositive cattle also might be identified after BoHV-5 infection. A study involving CpHV-1, another alphaherpesvirus related to BoHV-1, but more distantly than BoHV-5, revealed that some goats infected with CpHV-1 can be seropositive to BoHV-1 gE (Thiry et al., 2008). Since BoHV-1 is more similar to BoHV-5 than CpHV-1, the same situation could occur with BoHV-5 (Thiry et al., 2008).

Vaccines

Due to the sporadic frequency of detection and the restricted geographical distribution of BoHV-5 infections, few studies have focussed on the development of BoHV-5 vaccines. Furthermore, since BoHV-5 cases or outbreaks are reported in countries where there is a high prevalence of BoHV-1, efforts are focussed on the evaluation of the cross-protection induced by BoHV-1 vaccines against BoHV-5 infection. The evaluation of the efficacy of vaccines is further complicated by the difficulty in reproducing the clinical disease associated with BoHV-5 infections.

Bratanich et al. (1991) and Cascio et al. (1999) studied the cross-protection induced by BoHV-1 vaccines. Although they obtained a reduction in the period of viral shedding and the titre of virus excreted, their results could be misinterpreted because they did not reproduce the disease associated with BoHV-5. Another study showed that colostrum-acquired BoHV-1 antibodies prevent BoHV-5 induced neurological disease, but not BoHV-5 latency (Belknap et al., 1994).

The efficacy of a gE negative BoHV-1 vaccine was tested against BoHV-5 infection. This vaccine was not protective in rabbits or cattle (Silva et al., 2006; Spilki et al., 2004). A recent study reported the immunogenicity and efficacy of inactivated BoHV-1 and BoHV-5 vaccines against BoHV-5 disease (Del Médico Zajac et al., 2006). Both vaccines induced a similar humoral and cellular immune response. In addition, vaccinated animals were partially protected against BoHV-5 disease, since they showed a highly reduced viral excretion and less severe nervous signs compared with control animals.

Specific BoHV-5 vaccines remain at an experimental stage of development. The use of BoHV-1 vaccines is therefore considered to be the best option to protect against BoHV-5. However, several studies show that full protection against BoHV-5 infection is difficult to induce with a BoHV-1 vaccine. These results suggest that each BoHV-1 vaccine should be carefully tested for potential cross-protection against BoHV-5.

Conclusions

BoHV-5 is an alphaherpesvirus with a limited geographical distribution, since cases of BoHV-5 associated meningoencephalitis are commonly reported in South American countries, but only sporadically in other continents. However, since this virus is closely related to BoHV-1 and currently available serological tests do not differentiate antibodies against each virus, the true prevalence of BoHV-5 infection remains unknown. A refined molecular analysis of the strains in different continents could help to trace the spread

of the virus and explain the difference in these epidemiological situations. Furthermore, BoHV-5 may act as a confounder in BoHV-1 control programmes (Thiry et al., 2001).

The comparative analysis of BoHV-1 and BoHV-5 infections with regard to neuroinvasion and neurovirulence could provide interesting information about the neuropathogenesis of alphaherpesvirus infections, with a special relevance to HSV-1 encephalitis. The close similarity between BoHV-1 and BoHV-5 is unique in this respect.

References

Abril, C., Engels, M., Liman, A., Hilbe, M., Albini, S., Franchini, M., Suter, M., Ackermann, M., 2004. Both viral and host factors contribute to neurovirulence of bovine herpesviruses 1 and 5 in interferon receptor-deficient mice. Journal of Virology 78, 3644–3653.

Afonso, D.A., Ortega, L.S., Redondo, R.A., Trindade, Gde S., Barbosa-Stancioli, E.F., 2007. Characterization of field bovine herpesvirus samples using random amplified polymorphic DNA (RAPD). Journal of Virological Methods 140, 200–205.

Alegre, M., Nanni, M., Fondevila, N., 2001. Development of a multiplex polymerase chain reaction for the differentiation of bovine herpesvirus-1 and -5. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 48, 613–621.

Al-Mubarak, A., Chowdhury, S.I., 2004. In the absence of glycoprotein I (gI), gE determines bovine herpesvirus type 5 neuroinvasiveness and neurovirulence. Journal of Neurovirology 10, 233–243.

Ambagala, A.P.N., Gopinath, R.S., Srikumaran, S., 2004. Peptide transport activity of the transporter associated with antigen processing (TAP) is inhibited by an early protein of equine herpesvirus-1. Journal of General Virology 85, 349–353.

Ashbaugh, S.E., Thompson, K.E., Belknap, E.B., Schultheiss, P.C., Chowdhury, S., Collins, J.K., 1997. Specific detection of shedding and latency of bovine herpesvirus 1 and 5 using a nested polymerase chain reaction. Journal of Veterinary Diagnostic Investigation 9, 387–394.

Babiuk, L.A., van Drunen Littel-van den Hurk, S., Tikoo, S.K., 1996. Immunology of bovine herpesvirus 1 infection. Veterinary Microbiology 53, 31–42.

Bagust, T.J., 1972. Comparison of the biological, biophysical and antigenic properties of four strains of infectious bovine rhinotracheitis herpesvirus. Journal of Comparative Pathology 82, 365–374.

Bagust, T.J., Clark, L., 1972. Pathogenesis of meningo-encephalitis produced in calves by infectious bovine rhinotracheitis herpesvirus. Journal of Comparative Pathology 82, 375–383.

Bartha, A., Hajdu, G., Aldasy, P., Paczolay, G., 1969. Occurrence of encephalitis caused by infectious bovine rhinotracheitis virus in calves in Hungary. Acta Veterinaria Academiae Scientiarum Hungaricae 19, 145–151.

Belak, K., Kucsera, L., Ros, C., Kulcsar, G., Makranszki, L., Soos, T., Belak, S., 1999. Studies on the pathogenicity of bovine herpesvirus type 5 in sheep. Comparative Immunology, Microbiology and Infectious Diseases 22, 207–220.

Belknap, E.B., Collins, J.K., Ayers, V.K., Schultheiss, P.C., 1994. Experimental infection of neonatal calves with neurovirulent bovine herpesvirus type 1.3. Veterinary Pathology 31, 358–365.

Bottcher, S., Maresch, C., Granzow, H., Klupp, B., Peifke, J., Mettenleiter, T., 2008. Mutagenesis of active-site cysteine in the ubiquitin-specific protease contained in large tegument protein pUL36 of pseudorabies virus impairs viral replication in vitro and neuroinvasion in vivo. Journal of Virology 82, 6009–6016.

Brake, F., Studdert, M.J., 1985. Molecular epidemiology and pathogenesis of ruminant herpesviruses including bovine, buffalo and caprine herpesviruses l and bovine encephalitis herpesvirus. Australian Veterinary Journal 62, 331–334.

Bratanich, A.C., Sardi, S.I., Smitsaart, E.N., Schudel, A.A., 1991. Comparative studies of BHV-1 variants by in vivo–in vitro tests. Zentralblatt für Veterinarmedizin Reihe B 38, 41–48.

Carrillo, B.J., Pospischil, A., Dahme, E., 1983a. Pathology of a bovine viral necrotizing encephalitis in Argentina. Zentralblatt für Veterinarmedizin Reihe B 30, 161–168.

Carrillo, B.J., Ambrogi, A., Schudel, A.A., Vazquez, M., Dahme, E., Pospischil, A., 1983b. Meningoencephalitis caused by IBR virus in calves in Argentina. Zentralblatt für Veterinarmedizin Reihe B 30, 327–332.

Cascio, K.E., Belknap, E.B., Schultheiss, P.C., Ames, A.D., Collins, J.K., 1999. Encephalitis induced by bovine herpesvirus 5 and protection by prior vaccination or infection with bovine herpesvirus 1. Journal of Veterinary Diagnostic Investigation 11, 134–139.

Chowdhury, S.I., Lee, B.J., Mosier, D., Sur, J.H., Osorio, F.A., Kennedy, G., Weiss, M.L., 1997. Neuropathology of bovine herpesvirus type 5 (BHV-5) meningoencephalitis in a rabbit seizure model. Journal of Comparative Pathology 117, 295–310.

Chowdhury, S.I., Lee, B.J., Onderci, M., Weiss, M.L., Mosier, D., 2000a. Neurovirulence of glycoprotein C (gC)-deleted bovine herpesvirus type-5 (BHV-5) and BHV-5 expressing BHV-1 gC in a rabbit seizure model. Journal of Neurovirology 6, 284–295.

Chowdhury, S.I., Lee, B.J., Ozkul, A., Weiss, M.L., 2000b. Bovine herpesvirus 5 glycoprotein E is important for neuroinvasiveness and neurovirulence in the olfactory pathway of the rabbit. Journal of Virology 74, 2094–2106.

Chowdhury, S.I., Onderci, M., Bhattacharjee, P.S., Al-Mubarak, A., Weiss, M.L., Zhou, Y., 2002. Bovine herpesvirus 5 (BHV-5) Us9 is essential for BHV-5 neuropathogenesis. Journal of Virology 76, 3839–3851.

Claus, M.P., Alfieri, A.F., Folgueras-Flatschart, A.V., Wosiacki, S.R., Medici, K.C., Alfieri, A.A., 2005. Rapid detection and differentiation of bovine herpesvirus 1 and 5 glycoprotein C gene in clinical specimens by multiplex-PCR. Journal of Virological Methods 128, 183–188.

D'Arce, R.C., Almeida, R.S., Silva, T.C., Franco, A.C., Spilki, F., Roehe, P.M., Arns, C.W., 2002. Restriction endonuclease and monoclonal antibody analysis of Brazilian isolates of bovine herpesviruses types 1 and 5. Veterinary Microbiology 88, 315–324.

Del Médico Zajac, M.P., Puntel, M., Zamorano, P., Sadir, A.M., Romera, S.A., 2006. BHV-1 vaccine induces cross-protection against BHV-5 disease in cattle. Research in Veterinary Science 81, 327–334.

Del Médico Zajac, M.P., 2007. Herpesvirus bovino 5: estudio de la infección, la respuesta inmune y los recombinantes con herpesvirus bovino 1 en el hospedador natural. PhD Thesis, Buenos Aires, Argentina, 145 p.

Delhon, G., Moraes, M.P., Lu, Z., Afonso, C.L., Flores, E.F., Weiblen, R., Kutish, G.F., Rock, D.L., 2003. Genome of bovine herpesvirus 5. Journal of Virology 77, 10339–10347.

Denis, M., Kaashoek, M.J., van Oirschot, J.T., Pastoret, P.P., Thiry, E., 1994. Quantitative assessment of the specific CD4+ T lymphocyte proliferative response in bovine herpesvirus 1 immune cattle. Veterinary Immunology and Immunopathology 42, 275–286.

Diel, D.G., Almeida, S.R., Brum, M.C., Dezengrini, R., Weiblen, R., Flores, E.F., 2007. Acute and latent infection by bovine herpesvirus type 5 in experimentally infected goats. Veterinary Microbiology 121, 257–267.

D'Offay, J.M., Mock, R.E., Robert, W., Fulton, D.V.M., 1993. Isolation and characterization of encephalitic bovine herpesvirus type 1 isolates from cattle in North America. American Journal of Veterinary Research 54, 534–539.

Engels, M., Ackermann, M., 1996. Pathogenesis of ruminant herpesvirus infections. Veterinary Microbiology 53, 3–15.

French, A.L., 1962. A specific virus encephalitis in calves: isolation and characterization of the causal agent. Australian Veterinary Journal 38, 216–221.

Fuchs, M., Hubert, P., Detterer, J., Rziha, H.J., 1999. Detection of bovine herpesvirus type 1 in blood from naturally infected cattle by using a sensitive PCR that discriminates between wild-type virus and virus lacking glycoprotein E. Journal of Clinical Microbiology 37, 2498–2507.

Fuchs, W., Granzow, H., Klupp, B., Karger, A., Michael, K., Maresch, C., Klopfleisch, R., Mettenleiter, T., 2007. Relevance of the interaction between alphaherpesvirus UL3.5 and UL48 proteins for viral maturation and neuroinvasion. Journal of Virology 81, 9307–9318.

Gomes, L.I., Rocha, M.A., Souza, J.G., Costa, E.A., Barbosa-Stancioli, E.F., 2003. Bovine herpesvirus 5 (BoHV-5) in bull semen: amplification and sequence analysis of the US4 gene. Veterinary Research Communications 27, 495–504.

Gularte Quincozes, C., 2005. Prevalencia e fatores de risco asociados as infeccoes pelos herpesvirus bovino tipo 1 e 5 (BHV-1 e 5) e pelo virus da diarreia viral bovina (BVDV) nos rebanhos dos municipios de Santa Vitoria do Palmar e Chui. PhD Thesis, Rio Grande do Sul, Brasil, 103 p.

Hubner, S.O., Oliveira, A.P., Franco, A.C., Esteves, P.A., Silva, A.D., Spilki, F.R., Rijsewijk, F.A., Roehe, P.M., 2005. Experimental infection of calves with a gI, gE, US9 negative bovine herpesvirus type 5. Comparative Immunology, Microbiology and Infectious Diseases 28, 187–196.

Johnston, L.A.Y., Simmons, G.C., McGavin, M.D., 1962. A viral meningoencephalitis in calves. Australian Veterinary Journal 38, 207–215.

Keuser, V., Schynts, F., Detry, B., Collard, A., Robert, B., Vanderplasschen, A., Pastoret, P.P., Thiry, E., 2004. Improved antigenic methods for differential diagnosis of bovine, caprine, and cervine alphaherpesviruses related to bovine herpesvirus 1. Journal of Clinical Microbiology 42, 1228–1235.

Klopfleisch, R., Klupp, B., Fuchs, W., Kopp, M., Teifke, J., Mettenleiter, T., 2006. Influence of pseudorabies virus proteins on neuroinvasion and neurovirulence in mice. Journal of Virology 80, 5571–5576.

Liang, X., Chow, B., Raggo, C., Babiuk, L.A., 1996. Bovine herpesvirus 1 UL49.5 homolog gene encodes a novel viral envelope protein that forms a disulfide-linked complex with a second virion structural protein. Journal of Virology 70, 1448–1454.

Lindner, A., Ambrosius, H., Liebermann, H., 1993. Vergleichende Untersuchungen zum Nachweis von Antikörpern gegen BHV-5 mit dem Enzym-linked Immunosorbent Assay, Serumneutralisationstest, Zellassay und Immunfluoreszenztest. Deutsch Tierärztliche Wochenschrift 100, 440–442.

Mettenleiter, T.C., 2003. Pathogenesis of neurotropic herpesviruses: role of viral glycoproteins in neuroinvasion and transneuronal spread. Virus Research 92, 197–206.

Metzler, A.E., Schudel, A.A., Engels, M., 1986. Bovine herpesvirus 1: molecular and antigenic characteristics of variant viruses isolated from calves with neurological disease. Archives of Virology 87, 205–217.

Meyer, G., Lemaire, M., Ros, C., Belak, K., Gabriel, A., Cassart, D., Coignoul, F., Belak, S., Thiry, E., 2001. Comparative pathogenesis of acute and latent infections of calves with bovine herpesvirus types 1 and 5. Archives of Virology 146, 633–652.

Moretti, B., Orfei, Z., Mondino, G., Persechino, A., 1964. Infectious bovine rhinotracheitis, clinical observations and isolation of virus. Veterinaria Italiana 15, 676–702.

Muylkens, B., Thiry, J., Kirten, P., Schynts, F., Thiry, E., 2007. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Veterinary Research 38, 181–209.

Mweene, A.S., Okazaki, K., Kida, H., 1996. Detection of viral genome in non-neural tissues of cattle experimentally infected with bovine herpesvirus 1. Japanese Journal of Veterinary Research 44, 165–174.

Nugent, J., Birch-Machin, I., Smith, K.C., Mumford, J.A., Swann, Z., Newton, J.R., Bowden, R.J., Allen, G.P., Davis-Poynter, N., 2006. Analysis of equid herpesvirus 1 strain variation reveals a point Mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. Journal of Virology 80, 4047–4060.

Odeón, A.C., Spath, E.J.A., Paloma, E.J., Leunda, M.R., Fernandez Sainz, I.J., Perez, S.E., Kaiser, G.G., Draghi, M.G., Cetrá, B.M., Cano, A., 2001. Seroprevalencia de la diarrea viral bovina, herpesvirus bovino y virus sincicial respiratorio en Argentina. Revista de Medicina Veterinaria (Argentina) 82, 216–220.

Pastoret, P.P., Thiry, E., Brochier, B., Derboven, G., 1982. Bovid herpesvirus 1 infection of cattle: pathogenesis, latency, consequences of latency. Annals of Veterinary Research 13, 221–235.

Perez, S.E., Bretschneider, G., Leunda, M.R., Osorio, E.A., Flores, E.F., Odeon, A.C., 2002. Primary infection, latency, and reactivation of bovine herpesvirus type 5 in the bovine nervous system. Veterinary Pathology 39, 437–444.

Perez, S.E., Vagnozzi, A., Sur, J.H., Odriozola, E., Campero, C.M., Odeón, A.C., 2003. Análisis restrospectivo de casos con diagnóstico de necrosis cerebrocortical y su relación con herpesvirus bovino tipo 5. Revista Argentina de Microbiología 35, 69–73.

Reed, D.E., Bicknell, E.J., Bury, R.J., 1973. Systemic form of infectious bovine rhinotracheitis in young calves. Journal of the American Veterinary Medical Association 163, 753–755.

Riet-Correa, F., Vidor, T., Schild, A.L., Mendez, M.C., 1989. Meningoencefalite e necrose do cortex cerebral em bovinos causadas por herpes virus bovino-1. Pesquisa Veterinária Brasileira 9, 13–16.

Riet-Correa, G., Dutra Duarte, M., Barbosa, J.D., Chaves Oliveira, C.M., Duarte Cerqueira, V., de Farias Brito, M., Riet-Correa, F., 2006. Meningoencefalite e poliencefalomalacia causada por herpesvirus bovino-5 no Estado do Pará. Pesquisa Veterinária Brasileira 26, 44–46.

Rock, D., Lokensgard, J., Lewis, T., Kutish, G., 1992. Characterization of dexamethasone-induced reactivation of latent bovine herpesvirus 1. Journal of Virology 66, 2484–2490.

Roels, S., Charlier, G., Letellier, C., Meyer, G., Schynts, F., Kerkhofs, P., Thiry, E., Vanopdenbosch, E., 2000. Natural case of bovine herpesvirus 1 meningoencephalitis in an adult cow. Veterinary Record 146, 586–588.

Roizman, B., Desrosiers, R.C., Fleckenstein, B., Lopez, C., Minson, A.C., Studdert, M.J., 1992. The family Herpesviridae: an update. Archives of Virology 123, 425–449.

Roizman, B., Pellet, P.E., 2007. The family Herpesviridae: a brief introduction. In: Fields, B.N., Knipe, D.M., Howley, P.M., Chanock, R.M., Monath, T.P., Melnick, J.L., Roizman, B., Straus, S.E. (Eds.), Fields Virology, fourth ed. Lippincott Williams and Wilkins, Philadelphia, pp. 2480–2499.

Romera, S.A., Hilgers, L.A., Puntel, M., Zamorano, P.I., Alcon, V.L., Dus Santos, M.J., Blanco Viera, J., Borca, M.V., Sadir, A.M., 2001. Adjuvant effects of sulfolipo-cyclodextrin in a squalane-in-water and water-in-mineral oil emulsions for BHV-1 vaccines in cattle. Vaccine 19, 132–141.

Ros, C., Belak, S., 1999. Studies of genetic relationships between bovine, caprine, cervine, and rangiferine alphaherpesviruses and improved molecular methods for virus detection and identification. Journal of Clinical Microbiology 37, 1247–1253.

Ros, C., Belak, S., 2002. Characterization of the glycoprotein B gene from ruminant alphaherpesviruses. Virus Genes 24, 99–105.

Salvador, J.C., Lemos, R.A.A., Riet-Correa, F., Roehe, P.M., Osorio, A.R., 1998. Meningoencefalite em bovinos causada por herpesvirus bovino-5 no Mato Grosso do Sul e Sao Paulo. Pesquisa Veterinária Brasileira 18, 75–82.

Schudel, A.A., Carrillo, B.J., Wyler, R., Metzler, A.E., 1986. Infections of calves with antigenic variants of bovine herpesvirus type 1 (BHV-1) and neurological disease. Zentralblatt für Veterinarmedizin Reihe B 33, 303–310.

Schwyzer, M., Ackermann, M., 1996. Molecular virology of ruminant herpesviruses. Veterinary Microbiology 53, 17–29.

Schynts, F., McVoy, M.A., Meurens, F., Detry, B., Epstein, A.L., Thiry, E., 2003. The structures of bovine herpesvirus 1 virion and concatemeric DNA: implications for cleavage and packaging of herpesvirus genomes. Virology 314, 326–335.

Silva, A.D., Spilki, F.R., Franco, A.C., Esteves, P.A., Hubner, S.O., Driemeier, D., Oliveira, A.P., Rijsewijk, F., Roehe, P.M., 2006. Vaccination with a gE-negative bovine herpesvirus type 1 vaccine confers insufficient protection to a bovine herpesvirus type 5 challenge. Vaccine 24, 3313–3320.

Silva, A.M., Weiblen, R., Irigoyen, L.F., Roehe, P.M., Sur, H.J., Osorio, F.A., Flores, E.F., 1999. Experimental infection of sheep with bovine herpesvirus type-5 (BHV-5): acute and latent infection. Veterinary Microbiology 66, 89–99.

Silva, M.S., Brum, M.C., Loreto, E.L., Weiblen, R., Flores, E.F., 2007. Molecular and antigenic characterization of Brazilian bovine herpesvirus type 1 isolates recovered from the brain of cattle with neurological disease. Virus Research 129, 191–199.

Spilki, F.R., Silva, A.D., Hubner, S., Esteves, P.A., Franco, A.C., Driemeier, D., Roehe, P.M., 2004. Partial protection induced by a BHV-1 recombinant vaccine against challenge with BHV-5. Annals of the New York Academy of Sciences 1026, 247–250.

Studdert, M.J., 1989. Bovine encephalitis herpesvirus. Veterinary Record 125, 584.

Thiry, E., Ackermann, M., Banks, M., Belak, S., Campbell, I., Ek-Kommonen, C., Engels, M., Meyer, G., Reid, H., Ros, C., Six, A., 2001. Risk evaluation of cross-infection of cattle with ruminant alphaherpesviruses related to bovine herpesvirus type 1. In: Körber, R. (Ed.), Tagungsbroschüre, 3. International Symposium zur BHV-1-/BVD-Bekämpfung, Stendal, pp. 99–104.

Thiry, J., Tempesta, M., Camero, M., Tarsitano, E., Muylkens, B., Meurens, F., Thiry, E., Buonavoglia, C., 2007a. Clinical protection against caprine herpesvirus 1 genital infection by intranasal administration of a live attenuated glycoprotein E negative bovine herpesvirus 1 vaccine. BMC Veterinary Research 3, 33.

Thiry, J., Widen, F., Gregoire, F., Linden, A., Belak, S., Thiry, E., 2007b. Isolation and characterisation of a ruminant alphaherpesvirus closely related to bovine herpesvirus 1 in a free-ranging red deer. BMC Veterinary Research 3, 26.

Thiry, J., Saegerman, C., Chartier, C., Mercier, P., Keuser, V., Thiry, E., 2008. Serological evidence of caprine herpesvirus 1 infection in Mediterranean France. Veterinary Microbiology 128, 261–268.

Vogel, F.S., Caron, L., Flores, E.F., Weiblen, R., Winkelmann, E.R., Mayer, S.V., Bastos, R.G., 2003. Distribution of bovine herpesvirus type 5 DNA in the central nervous systems of latently, experimentally infected calves. Journal of Clinical Microbiology 41, 4512–4520.

Wellenberg, G.J., Mars, M.H., Van Oirschot, J.T., 2001. Antibodies against bovine herpesvirus (BHV) 5 may be differentiated from antibodies against BHV1 in a BHV1 glycoprotein E blocking ELISA. Veterinary Microbiology 78, 79–84.

Winkler, M.T., Doster, A., Jones, C., 2000. Persistence and reactivation of bovine herpesvirus 1 in the tonsils of latently infected calves. Journal of Virology 74, 5337–5346.