Vaccination with a gE-negative bovine herpesvirus type 1 vaccine confers insufficient protection to a bovine herpesvirus type 5 challenge

Alessandra D. Silva\textsuperscript{a,b,}\textsuperscript{*}, Fernando R. Spilki\textsuperscript{b}, Ana Cláudia Franco\textsuperscript{a},
Paulo A. Esteves\textsuperscript{b,c}, Silvia O. Hübner\textsuperscript{a}, David Driemeier\textsuperscript{d},
Anna Paula Oliveira\textsuperscript{a,e}, Frans Rijsewijk\textsuperscript{f}, Paulo M. Roche\textsuperscript{a,e}

\textsuperscript{a} Equipe de Virologia, FEPAGRO Saúde Animal – Instituto de Pesquisas Veterinárias Desidério Finamor (CPVDF), Estrada do Conde 6000, Eldorado do Sul, RS, Brazil
\textsuperscript{b} Programa de Pós-Graduação em Ciências Veterinárias (PPGCV), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
\textsuperscript{c} Empresa Brasileira de Pesquisa Agropecuária (Embrapa Aves e Suínos), BR 153, Km 110, Vila Tamanduá, Concórdia, SC, Brazil
\textsuperscript{d} Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bentor Gonçalves 9890, Porto Alegre, RS, Brazil
\textsuperscript{e} Instituto de Ciências Básicas da Saúde, UFRGS, Rua Samurango Leite, 500, CEP: 90050-170, Porto Alegre, RS, Brazil
\textsuperscript{f} Animal Sciences Group, Postbus 65, 8200 AB, Lelystad, The Netherlands

Received 6 July 2005; received in revised form 6 January 2006; accepted 10 January 2006
Available online 25 January 2006

Abstract

In the present study, cross-protection to bovine herpesvirus type 5 (BHV-5) induced by bovine herpesvirus type 1 (BHV-1) vaccination was examined following inoculation of rabbits and calves with a glycoprotein E (gE)-negative BHV-1 vaccine and subsequent challenge with BHV-5. Rabbits (n = 5) and calves (n = 8) were vaccinated [five rabbits intranasally (IN), four calves IN and four intramuscularly (IM)] with 7.1 log\textsubscript{10}median tissue culture infective dose (TCID\textsubscript{50}) of the BHV-1 vaccine. Rabbits and calves were challenged IN [rabbits 2 weeks post-vaccination (pv); calves 5 weeks pv] with 9.1 log\textsubscript{10} TCID\textsubscript{50} of BHV-5. Two out of five vaccinated rabbits died after challenge with typical BHV-5 disease, as did 3/5 non-vaccinated controls. In calves, 4/8 vaccinated animals displayed mild signs of disease, whereas 6/6 non-vaccinated controls developed signs of disease, so severe that 2/6 had to be killed. Besides, nasal virus shedding post-challenge was not reduced by vaccination. At necropsy, on day 21 post-challenge, typical BHV-5 lesions were evident in brain tissues of both vaccinated and non-vaccinated calves. Dexametasone administration at 180 days post-infection did not reactivate clinical signs despite BHV-5 shedding in nasal secretions of both vaccinated and non-vaccinated calves. These results show that the BHV-1 vaccine evaluated here did not confer protection to BHV-5 in rabbits. In calves, BHV-1 vaccination did confer some protection to BHV-5 induced clinical disease, but it did not prevent infection and had no effect on nasal virus shedding or on the development of encephalitic lesions.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Bovine herpesvirus type 1; Bovine herpesvirus type 5; Immunization

1. Introduction

Bovine herpesvirus type 5 (BHV-5), an Alphaherpesvirus closely related to infectious bovine rhinotracheitis virus (bovine herpesvirus type 1; BHV-1), is the causative agent of bovine herpesvirus encephalitis [1]. Clinically apparent BHV-5 infections induce a necrotizing meningoencephalitis, although signs of respiratory disease may also be present [2–5]. Like BHV-1, animals that recover from primary BHV-5 infection develop a latent infection; latent virus has been found in several areas of the brain [6] and within neurons of the trigeminal ganglion [7].

BHV-5 is very similar to BHV-1, as reflected by the level of amino acid identity in their protein repertoires (average 82%) [8,9]. The highest similarity to BHV-1 products (>95%...
amino acid identity) is found in proteins involved in viral DNA replication and processing (UL5, UL15, UL29 and UL39) and in virion proteins (UL14, UL19, UL48 and US6) [9]. Several genes are apparently involved in BHV-1 and BHV-5 virulence. Among these, the genes coding for proteins glycoprotein E (gE) and glycoprotein I (gI) have been particularly incriminated in virulence in several studies [10–12]. Glycoprotein E is a non-essential glycoprotein which is predominantly found complexed with glycoprotein I in different alphaherpesviruses [13]. Such complex was shown to facilitate cell-to-cell spread as well as the anterograde axonal transport in neurons [13–17]. Thus, the lack of the gI-gE complex may impair significantly the anterograde transport of the virus in axons, limiting its spread, thereby reducing pathogenicity. In rabbits, a gE gene-deleted (gE−) BHV-5 replicated and spread significantly less in the central nervous system (CNS) and induced significantly less neurological signs than the wild type virus [5,10,18]. Besides, in some herpesviruses such as herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV), the gE−gI complex functions as receptor for the Fc domain of immunoglobulin G and consequently may play a role in evasion of the host’s immune responses [10–19].

BHV-5 is a growing concern for cattle farming in Southern Brazil and Argentina [22–26]. Despite the extensive serological cross-reactions between BHV-5 and BHV-1 [27,28], it is not clear whether BHV-1 vaccines would cross-protect cattle against BHV-5-induced encephalitis [29,30]. Under field conditions, veterinarians have recommended vaccination with BHV-1 vaccines in attempting to control outbreaks of BHV-5 encephalitis [31]. However, it is not known whether such procedure would actually be efficacious. Previous experiments with conventional live BHV-1 vaccines have revealed some degree of protection, but those have been difficult to quantitative, particularly in view of the difficulties in reproducing BHV-5 disease [30]. Also in function of such difficulty (as well as costs), rabbits have often been used as models for BHV-5 infections [31,32]. In previous studies, we described the construction of a recombinant vaccine, engineered from an autochtonous (Brazilian) BHV-1 strain [33], from which the gene coding for glycoprotein E-deficient (gE−) was deleted. Such vaccine allies the benefits of attenuation conferred by the removal of gE [34] with the fact that the serological response induced by vaccination can be differentiated from that of wild type virus with an immunoassay that targets anti-gE antibodies. The gE− vaccine was proved highly effective in vaccination/challenge experiments, where it was shown to significantly reduce clinical signs of disease and virus shedding from vaccinated/challenged calves [34]. In order to check whether BHV-1 vaccination with such a vaccine would induce cross-protection to BHV-5 infections, in the present study rabbits were vaccinated with the gE− vaccine [33] and subsequently challenged with a highly virulent BHV-5 strain. The experiment was subsequently extended to cattle, the actual target species.

2. Materials and methods

2.1. Cells and viruses

Virus multiplication, quantitation and isolation from tissues as well as neutralization assays were performed on Madin Darby bovine kidney cells (MDBK, ATCC, CCL-22). Cells were routinely maintained in Eagle’s minimal essential medium (E-MEM) supplemented with 6% fetal calf serum (FCS, Nutricell) and 2 mg/L enrofloxacin (Baytril, Bayer). Cells were maintained and multiplied following standard procedures [35].

The recombinant vaccine used for immunization of rabbits and calves was derived from a Brazilian wild-type BHV-1.2a from which the glycoprotein E gene was deleted [33]. The BHV-5 used for challenge was the wild type strain EVI 88/95. Such strain is a typical representative of the majority of BHV-5 isolates circulating in Brazil and has been characterized in a number of previous studies [36–42]. In addition, strain BHV-1 strain EVI 123/98 [36] was multiplied as above and used in neutralization tests (see below). Such strain has previously shown to recognize most BHV-1-positive sera in previous tests in our laboratory (data not shown).

2.2. Vaccination and challenge of rabbits

Fifteen rabbits of mixed breeds, 30-day-old, were kept in isolation units with food and water ad libitum. The rabbits were divided in three groups: in the first, five animals were vaccinated with 7.1 log10 median tissue culture infective dose (TCID50) of the BHV-1 vaccine 265 gE−[33], divided in two volumes of 0.8 mL applied into each nostril. A second group of five rabbits was mock vaccinated with sterile E-MEM to be further used as challenge controls. A third group of five rabbits was kept as non-vaccinated, unchallenged controls. For infection, vaccination and challenge, rabbits were previously anaesthetized with 2 mg of tiletamine/zolazepam (Zoletil, Virbac). Infections were made directly into the paranasal sinuses, as previously described [43]. Clinical investigations included weighing, measurement of rectal temperature, and search for sneezing, coughing, nasal and ocular secretions, depression (anorexia, apathy) and neurological signs (convulsions, tremors, coma). Blood samples for serological examinations were collected on days 0, 7 and 14 post-vaccination (pv).

On day 14 pv, the rabbits on the first and second groups were challenged with a same volume of a suspensions containing 9.1 log10 TCID50 of BHV-5 strain EVI 88/95 following the same inoculation protocol as above. The rabbits were again monitored daily in search for clinical signs and blood samples collected on days 0, 7 and 14 post-challenge (pc). All animals that died were necropsied. Brains and lungs were collected for further studies.

2.3. Vaccination and challenge of calves

Sixteen calves of mixed breeds, 3–4-month-old, seronegative for both BHV-1 and BHV-5, were divided in three...
In the first group, eight calves were vaccinated [four intranasally (IN) and four intramuscularly (IM)] with 7.1 log10 TCID50 of the BHV-1 vaccine (total volume 2 mL) applied either intranasally in the right nostril or intramuscularly on the right side of the neck. Six other non-vaccinated calves were kept to be later used as challenge controls. Two additional calves were kept as non-vaccinated, non-challenged controls. Thirty-five days later, calves (vaccinated and challenge controls) were challenged with 9.1 log10 TCID50 of BHV-5 strain EVI 88/95 applied intranasally as described above. The animals were monitored daily until day 21 post-challenge, when two calves in each group (two vaccinated IN, two vaccinated IM, two mock vaccinated and challenged) plus one of the negative control calves were killed and necropsied. The remaining seven calves were 60 days later submitted to corticosteroid administration (0.2 mg/kg of body weight per day of dexamethasone for five consecutive days) in attempting to induce virus reactivation and kept in observation for another 14 days. Nasal swabs were collected daily from days 0 to day 14 post-challenge and day 0 to day 14 post-application of dexamethasone and 5 days before beginning of administration of dexamethasone. Swabs were immersed in 1 mL of sterile E-MEM supplemented with 200 U/mL penicillin, 200 μg/mL streptomycin and 5 μg/mL Amphotericin B. Samples were stored at −70 °C until processing.

All procedures involving animal care, handling and experiments were performed under veterinary supervision and according to the recommendations of the Brazilian Committee on Animal Experimentation (COBEA; law no. 6.638 of May 8th, 1979).

2.4. Virus isolation

Fragments of distinct regions of brains of rabbits and calves (olfactory bulb, anterior cortex, posterior cortex, diencephalon, cerebellum and pons) as well as portions of lungs were submitted to virus isolation [31]. The tissues were homogenized with sterile sand at 10% (w/v) with E-MEM supplemented with antibiotics (as above) and clarified at 2500 × g for 20 min at 4 °C. Volumes of 0.2 mL of each supernatant were inoculated onto MDBK monolayers prepared on 24-well plates. Infected cultures were checked for the presence of virus for 7 days. All negative cultures were frozen at −70 °C, thawed and passaged once more on fresh MDBK monolayers. When viral ECP was detected, 10-fold dilutions of the original suspensions were titrated on MDBK cells. Recovered viruses were titrated and its identity confirmed by an immunoperoxidase monolayer assay (IPMA) as described below. Infectious titre were determined, calculated and expressed as log10 TCID50 per 50 μL [44] after 72 h of incubation at 37 °C.

2.5. Immunoperoxidase monolayer assay

Virus recovered from tissues were inoculated into 96-well plates with pre-formed MDBK monolayers, fixed in 4% paraformaldehyde and stained in an immunoperoxidase monolayer assay as previously described [45], with an anti-BHV-1 (11H6)[36,42] or anti-BHV-5 (2C3) [36,37] monoclonal antibody (Mab) as primary antibody.

2.6. Serum neutralization tests

Neutralizing antibodies in serum samples were examined in a varying serum-constant virus neutralization (SN) assay [46], with two-fold dilutions of serum against 100 TCID50 of either BHV-1 strain EVI 123/98 or BHV-5 strain EVI 88/95. Antibody titre were expressed as the reciprocal of the highest serum dilution that prevented the development of cytopathic effect (CPE) after 72 h of incubation at 37 °C.

2.7. Histopathology

Tissues for histological examination were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with haematoxylin–eosin (H&E) following routine protocols [47].

2.8. Statistical analysis

Statistical analysis was performed using the χ²-test, with α = 0.05 for death between different groups. The data on viral excretion and serological status was evaluated by analysis of variance (ANOVA). The least significant difference for p = 0.05 was determined. Comparisons were made from day-to-day within the groups and between groups. Mean survivor rates and intervals between inoculation and death among groups were compared with the Fisher’s exact test. Statistical analysis was performed with Data Analysis Supplement for Excel TM (Office System 2003 Windows TM, Microsoft Corp., Seattle, USA). The term “significant” (statistically significant) means p ≤ 0.05.

3. Results

3.1. Vaccination and challenge of rabbits

No signs of disease were observed during the 14 days from vaccination up to challenge. After challenge, two out of the five (40%) vaccinated rabbits died in the acute phase of infection (on days 11 and 14 pc). Characteristic clinical signs of BHV-5 infection were observed in vaccinated rabbits that died (pyrexia, apathy, anorexia, cough, sneezing, convulsions with muscular movements starting around the nose and mouth and spreading sequentially to the muscles of the neck, walking in circles, incoordination, paddling, opisthotonus, tremors, coma and death). In non-vaccinated rabbits submitted to challenge, three out of five (60%) rabbits developed characteristic signs of BHV-5 infection and were euthanatized or died between days 7 and 14 pc (Table 1).
Table 1

Major clinico-pathological findings on rabbits vaccinated with a BHV-1 vaccine (or non-vaccinated controls) submitted to challenge with BHV-5

<table>
<thead>
<tr>
<th>Group</th>
<th>Clinical findings</th>
<th>Macroscopical findings</th>
<th>Histopathological findings</th>
<th>Outcome of acute infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>Tremors, seizures, coma and death</td>
<td>Lung: no alterations</td>
<td>Lung: no alterations; brain: diffuse microgliaosis, perivascular cell cuffing, neuronophagia and meningitis</td>
<td>2/5 animals died on days 11 and 14 post-challenge</td>
</tr>
<tr>
<td>Non-vaccinated</td>
<td>Respiratory distress, tremors, seizures, coma and death</td>
<td>Lung: diffuse haemorrhagic pneumonia</td>
<td>Lungs: purulent interstitial pneumonia; brain: diffuse microgliosis, perivascular cell cuffing, neuronophagia and meningitis</td>
<td>3/5 animals died between days 7 and 14 post-challenge</td>
</tr>
</tbody>
</table>

*a Number of affected rabbits/number of inoculated rabbits.

The onset of clinical disease was affected by vaccination. Two animals on the vaccinated group (2/5) had a delay (11 days pc) in the onset of clinical signs when compared to non-vaccinated rabbits (3/5; 7 days pc). However, such delay was not statistically significant on the Fisher’s test. Histological examination of brain sections of all animals (vaccinated and non-vaccinated) that were necropsied revealed diffuse microgliosis, associated perivascular cell cuffing, neuronophagia and meningitis (Table 1).

BHV-5 was recovered from different regions of brains of the vaccinated rabbits, particularly from the anterior cortices and diencephalon, to titres ranging from 0.8 to 2.05 log10 TCID50/mL (Table 2). In lung tissues, no virus was recovered from vaccinated rabbits (Table 2) whereas non-vaccinated rabbits had virus in lung tissues (albeit to low titres). These presented a diffuse multifocal hemorrhagic pneumonia, confirmed by histopathology (Table 1).

In the brain, the lesions observed in vaccinated rabbits were undistinguishable from those seen in non-vaccinated ones (Table 1). The surviving animals from both vaccinated and non-vaccinated groups were kept in observation for natural reactivation of latency. All rabbits displayed clinically apparent reactivation and died with characteristic clinical signs of BHV-5 disease between days 45 and 120 after challenge. All rabbits displayed clinical signs of BHV-5 disease between days 45 and 120 after challenge. After challenge, between days 3 and 11 pc, mild clinical signs of respiratory disease (nasal discharge, sneezing, coughing, and forced respiration) and depression of low magnitude (apathy, hypersalivation, incoordination) were evident in 4/8 vaccinated calves, as well as in 6/6 non-vaccinated/challenge calves. Meanwhile, other four out of the eight vaccinated calves showed no clinical signs throughout the experiments. On day 14 after vaccination (date of challenge), the rabbits on the non-vaccinated group remained seronegative, whereas vaccinated ones had low levels of virus-neutralizing antibody (VNAbs), with titres ranging from 2 to 4. The vaccinated rabbits, on day 7 pc, developed a strong secondary response with VNAbs titres of up to 32, whereas those on the non-vaccinated group remained seronegative. On day 14 pc, VNAbs titres on the surviving vaccinated rabbits ranged from 1.8 to 2.05 log10 TCID50/mL, whereas those on the non-vaccinated group had titres to 4. No significant differences in VNAbs (equal to or greater than four-fold) were detected in relation to the virus type (either BHV-1 or BHV-5) used in neutralization assays (Fig. 1).

Table 2

BHV-5 isolation from organs of rabbits vaccinated with a BHV-1 vaccine (or non-vaccinated controls) after BHV-5 challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Virus isolation after BHV-5 challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lungs</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>--*</td>
</tr>
<tr>
<td>Non-vaccinated</td>
<td>++</td>
</tr>
</tbody>
</table>

* Negative.

+++ = Infectious virus titres ranging from 0.8 to 1.3 log10 tissue culture infective doses per gram of tissue (TCID50); ++ = 1.3–1.8 log10 TCID50; +++ = 1.8–2.05 log10 TCID50; ++++ = greater than 2.05 log10 TCID50.

3.2. Vaccination and challenge of calves

Neither vaccinated nor non-vaccinated and control calves displayed any clinical signs during the 35 days from vaccination up to the date of challenge. After challenge, between days 3 and 11 pc, mild clinical signs of respiratory disease (nasal discharge, sneezing, coughing, and forced respiration) and depression of low magnitude (apathy, hypersalivation, incoordination) were evident in 4/8 vaccinated calves, as well as in 6/6 non-vaccinated/challenge calves. Meanwhile, other four out of the eight vaccinated calves showed no clinical signs throughout the experiments. BHV-5 virus shedding was detected in nasal secretions of all vaccinated and non-vaccinated calves up to day 14 pc. Yet in both groups, infectious virus titres in nasal secretions reached 4 log10 TCID50/mL by day 9 pc, and from then on tended to decrease up until day 14 pc, when sampling was discontinued (Fig. 2). Two non-vaccinated calves were more...
Fig. 1. Virus neutralizing antibody (VNAb) titres to (a) BHV-1 and (b) BHV-5 in sera of rabbits vaccinated with BHV-1 vaccine (black squares), non-vaccinated (black triangles) and controls (empty circles), submitted to challenge with BHV-5. Arrow points to date of challenge (14 days post-vaccination).

Fig. 2. BHV-5 virus shedding in nasal secretions of calves: (a) after BHV-5 challenge and (b) from the start of dexametasone administration (day “0” corresponds to day 120 post-vaccination). Empty losenges: calves vaccinated intranasally; empty squares: calves vaccinated intramuscularly; black triangles: non-vaccinated controls. Titres expressed in log10 TCID50/mL. Bars = standard deviation. Horizontal bar represents period of dexametasone administration.

Fig. 3. Neutralizing antibody titres to BHV-1 (a) and BHV-5 (b) after vaccination with BHV-1 vaccine, challenge with BHV-5 and during reactivation attempts in calves vaccinated intranasally (black triangle), intramuscularly (empty squares) and non-vaccinated (black circles). Arrow points to date of challenge (35 days post-vaccination) and date of the beginning of dexametasone administration (120 days post-vaccination).

severely affected and displayed more pronounced signs of neurological disease after challenge (hypersalivation profuse, teeth chewing, recumbency, incoordination, difficulty to stand, tremors). These calves were euthanatized in extremis, one on day 7 and another on day 11 pc. From these, infectious virus was recovered from the frontal cortex, pons, hippocampus, cerebellum and trigeminal ganglia.

On day 21 pc, four vaccinated calves (two calves that displayed depression of low magnitude and two with no evident clinical signs) and two non-vaccinated/challenged calves were necropsied. Post-mortem examinations revealed that all necropsied calves from both groups had typical BHV-5 lesions in the brain, with foci of malacia and sinking areas on the frontal, parietal and temporal lobes. Mononuclear meningoencephalitis was a consistent microscopical finding. However, infectious virus was not recovered from these animals. Calves on the negative control group remained healthy, did not shed virus in nasal secretions and remained seronegative throughout the experiments.

Serological examination confirmed that all calves were seronegative for both BHV-1 and BHV-5 virus at the beginning of the experiments. Calves (vaccinated and non-vaccinated) developed antibodies to BHV-1 and BHV-5, with no significant differences in VNAb titres to both virus types. At challenge (day 35 after vaccination), VNAb titres were above 32 in all vaccinated calves, regardless of the route (IN or IM) used for vaccination (Fig. 3). After challenge, a small rise in VNAbs was detected on day 21 pc (that is, day 56 pv). Testing for antibodies was discontinued by day 115 pc.
development of brain lesions in calves after challenge. However, nasal BHV-5 shedding post-challenge was not reduced; limited protection was attained following BHV-1 immunization. Thus, in vaccinated calves, only cross-protection on the actual target species to allow a conservative view on the subject. Hence, in vaccinated calves, only cross-protection on the actual target species to allow a conservative view on the subject. Consequently, BHV-5 infection was reported [30]. However, in that study, protection was not fully accessed in view of the difficulties in reproducing experimental BHV-5 encephalitis [30]. The authors suggested that vaccination with BHV-1 vaccines could protect against BHV-5 encephalitis, despite the absence of clinical signs or major macroscopical lesions in controls [30]. Here, it was possible to demonstrate that insufficient protection to BHV-5 challenge was conferred by BHV-1 vaccination, when vaccinated calves were submitted to challenge with virulent BHV-5. Despite some reduction in the severity of lesions associated to BHV-5 infection, it became clear that BHV-1 vaccination is not satisfactory for fully preventing BHV-5 induced disease. Undoubtedly, the data presented here refers to the particular vaccine [33,34] and experimental conditions employed in the present study. The behaviour of other BHV-1 vaccines in the induction of BHV-5 cross-protection should be examined to avoid undue extrapolations.

It must be stressed here that the infective doses of BHV-5 virus used for challenge in the experiments conducted here were quite high. However, this was purposely so in order to ensure the onset of clinically apparent disease in infected calves. Clearly, it is unlikely that cattle would become infected with such amount of virus in nature. Nevertheless, experimental infection of cattle with lower infective virus doses did not warrant the onset of clinical disease in all infected calves (data not shown). Difficulties in reproducing experimental BHV-5 disease have also been experienced, as mentioned above [30] what highlights the differences between natural and experimental reproduction of disease.

In summary, the results obtained here indicate that protection to challenge was not fully achieved by vaccination of calves with a BHV-1 live, gE-deficient vaccine. Future studies must be conducted in order to develop type-specific vaccine to control BHV-5 infections.
Acknowledgements

We thank Dr. L. Lovato, Dr. C. Canal and Dr. A. Simonetti for critically reviewing the manuscript. A.D. Silva and P.A. Esteves are students from the Programa de Pós-Graduação em Ciências Veterinárias da Faculdade de Vet-
erinárias (PPGCV/UFRGS/Brazil) grantees of CAPES. P.M. Roehe is a CNPq 1A research fellow. Work supported by CNPq, PRONEX, FAPERGS and CAPES.

References

[1] Murphy FA, Gibbs EP, Hertert MC, Studdert MJ. Veterinary virol-


[5] Moraes MP, Lu Z, Afonso CL, et al. The putative gJ of herpes simplex type 1 lacking glycoprotein gE, gI, gJ, or the putative gI is important for BoHV-5 neurovirulence. J Virol 2004;78(9):4806–16.


[7] Wellenberg GJ, Mars MH, Oorschot JT. Antibodies against bovine herpesvirus (BHV) 5 may be differentiated from antibodies against BHV1 in a BHV1 glycoprotein E blocking ELISA. Vet Microbiol 2001;78:79–84.


[9] Wellenberg GJ, Mars MH, Oorschot JT. Antibodies against bovine herpesvirus 5 may be differentiated from antibodies against BHV1 in a BHV1 glycoprotein E blocking ELISA. Vet Microbiol 2001;78:79–84.


[14] Moraes MP, Lu Z, Afonso CL, et al. The putative gJ of herpes simplex type 1 lacking glycoprotein gE, gI, gJ, or the putative gI is important for BoHV-5 neurovirulence. J Virol 2004;78(9):4806–16.

[15] Murphy FA, Gibbs EP, Horzinek MC, Studdert MJ. Veterinary virol-


