

# Detection of bovine herpesvirus-1 infection in breeding bull semen by virus isolation and polymerase chain reaction

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## Summary

Serum samples from 51 apparently healthy breeding bulls were screened for bovine herpesvirus-1 (BHV-1) antibodies using an avidin-biotin enzyme-linked immunosorbent assay, revealing a sero-positive prevalence rate of 45.09%. Semen samples were then collected from 12 of the sero-positive and 12 of the sero-negative bulls and tested for BHV-1 antigen using both a virus isolation assay and a polymerase chain reaction (PCR) assay; PCR was applied to detect BHV-1 deoxyribonucleic acid by using primers selected from the relatively conserved sequence of the gI glycoprotein gene to amplify a 468 base pair fragment. The PCR-amplified products were confirmed as BHV-1 by restriction enzyme, *Dde* I, which produced fragments of predictable sizes, namely 340 and 128 base pairs. Positive virus isolation test results, confirmed by virus neutralisation, found BHV-1 antigen in the semen of five sero-positive and six sero-negative bulls. In comparison, positive PCR results found BHV-1 genome in the semen of six sero-positive and eight sero-negative bulls. From the 24 semen samples tested, 14 were shown to be positive by PCR and 11 by virus isolation. The sensitivity and specificity of virus isolation were 57.14% and 70% respectively, and were significantly lower than PCR. In the semen samples taken from sero-negative bulls, BHV-1 was detected more often by PCR methods than by virus-isolation, suggesting that PCR is a more sensitive method for BHV-1 screening in bulls.

## Keywords

Bovine herpesvirus-1 – Breeding bulls – Polymerase chain reaction – Semen – Virus isolation.

## Introduction

Bovine herpesvirus-1 (BHV-1), the causative agent of infectious bovine rhinotracheitis (IBR), is considered to be the most common viral pathogen found in bovine semen (9). BHV-1 is responsible for a variety of clinical conditions in cattle and buffaloes, including pustular vulvovaginitis, abortion, mastitis, balanoposthitis of bulls, infertility, tracheitis, conjunctivitis–keratoconjunctivitis, encephalitis and fatal disease in newborn calves, and thus causes great economic losses to the livestock industry

(2, 9). Bovine herpesvirus-1 infection was first reported in India by Mehrotra *et al.* (12), and various workers have since reported the widespread seroprevalence and isolation of the virus, which they have isolated in different parts of the country (20, 22). The infection has serious economic implications for India, which is emerging as the world's biggest milk producer and has the world's largest cattle and buffalo population.

The latent virus may be established in the nerve ganglia of infected but clinically normal animals after primary infection despite the development of neutralising antibody

(18). Vaccines, although capable of preventing clinical disease, are unable to prevent the establishment of latency (30). Control by vaccination may therefore be contraindicated in bulls producing semen for export (3) due to the risk of spreading the disease if the latent virus is present. Periodic reactivation of latent BHV-1 has been shown to be associated with stress conditions or corticosteroid treatment (1, 5). The virus is excreted through secretions (nasal and ocular), and is present in the placenta of aborted animals and semen. Bovine semen is stored and handled in conditions that are ideal for preserving the viral pathogen, so contaminated semen presents a potential threat to the cattle industry: BHV-1 can spread through artificial insemination (AI), causing a variety of genital tract disorders such as endometritis, infertility and abortion (4, 6).

Although the risk of transmission of the virus can never be entirely eliminated, where semen is exported international trade regulations require certification that bulls at AI centres are BHV-1 sero-negative. However, attempts to eliminate the virus and maintain sero-negative bulls in AI centres in countries where there is widespread seroprevalence of BHV-1 may prove difficult in practice. Furthermore, demands for semen from bulls that are genetically outstanding and valuable but are also BHV-1 sero-positive (i.e. latently infected) dictate the alternative approach of monitoring all semen collections for the presence of virus, in order to control the spread of the disease (3). The maintenance of such semen-processing centres for AI purposes requires vigilant hygienic and regulatory measures supported by routine diagnostic tests that can detect BHV-1 in biological clinical samples quickly, economically and reliably. The present study was undertaken in order to evaluate different laboratory procedures for effective diagnosis of BHV-1 infection in breeding bulls. These procedures were: avidin-biotin enzyme-linked immunosorbent assay (A-B ELISA), virus isolation and polymerase chain reaction (PCR).

## Materials and methods

### Virus

Bovine herpesvirus-1 isolate IBR 216 II batch No. 9/98 (Passage No. 30) was obtained from the Indian Veterinary Research Institute, Izatnagar, India, and propagated in Madin Darby bovine kidney (MDBK) cells (obtained from the National Centre for Cell Science, Pune, India) in the presence of Eagle's minimum essential medium containing 2% foetal bovine serum.

### Collection of serum and semen samples

A total of 51 serum samples were collected from apparently healthy breeding bulls on two different organised farms in

Punjab state, India. Neat semen samples were collected simultaneously from the same bulls and stored at  $-70^{\circ}\text{C}$  until tested.

### Detection of antibodies to bovine herpesvirus-1

An A-B ELISA kit procured from the Project Directorate, Animal Disease Monitoring and Surveillance, Bangalore, India, was used to screen the serum samples for the presence of BHV-1 specific antibodies.

### Production of bovine herpesvirus-1 antiserum

Four injections of density gradient purified standard BHV-1 antigen (the first injection with Freund's complete adjuvant and the others with Freund's incomplete adjuvant) were given to four rabbits subcutaneously at weekly intervals. One week after the third injection, a blood sample was taken from ear veins to check for the presence of BHV-1 antibody. One week after the last injection, serum was collected and adsorbed overnight at  $4^{\circ}\text{C}$  on an MDBK cell monolayer to remove non-specific antibodies and clarified by centrifuge (6,000 rpm for ten minutes) for further use.

### Virus isolation from semen samples of sero-positive and sero-negative bulls

Semen samples from 24 different bulls (12 sero-positive and 12 sero-negative), diluted 1:10 in growth medium, were processed and inoculated separately onto the confluent MDBK cell monolayer, following the procedure described in the World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (15). The virus isolation test was considered to show that samples were BHV-1 negative if no cytopathic effect (CPE) was obtained after three passages.

Virus isolates that showed a CPE were subjected to the  $\alpha$ -method of virus neutralisation test, as recommended by the OIE, to confirm the isolates as BHV-1. The virus titre, neutralisation titre and neutralisation index in terms of tissue culture infective dose 50 (TCID<sub>50</sub>) were calculated for each sample, using the method described by Reed and Muench (19).

### Extraction of viral deoxyribonucleic acid for polymerase chain reaction assay

The split-sample method was used in PCR: a duplicate of each sample was spiked with aliquots of virus suspension (titre  $10^{-6}$  TCID<sub>50</sub> BHV-1/0.1 ml) and tested in parallel with the control sample. Viral deoxyribonucleic acid (DNA) was

extracted from the raw semen samples stored at  $-70^{\circ}\text{C}$ , cell culture passaged isolates and spiked semen samples by the phenol/chloroform/isoamyl-alcohol extraction method. Spermatozoa-free supernatant was obtained by mixing and centrifuging the samples with an equal volume of maintenance medium. This supernatant was treated with sodium dodecyl sulphate (0.2%) and proteinase K (250  $\mu\text{g}/\text{ml}$ ). The mixture was incubated at  $56^{\circ}\text{C}$  for one hour in a water bath, then mixed with an equal volume of a mixture of phenol, chloroform and isoamyl alcohol (25:24:1) and centrifuged at 14,000 rpm for 15 min. The aqueous fraction was subjected to one more cycle of phenol/chloroform/isoamyl-alcohol treatment followed by one cycle of chloroform/isoamyl-alcohol (24:1) treatment. Finally, after overnight incubation at  $-20^{\circ}\text{C}$ , the DNA was precipitated with an equal volume of sodium acetate (3.0 mol, pH 5.5) and isopropanol by centrifuge (15 m, 14,000 rpm). The DNA pellet was washed with 500  $\mu\text{l}$  of 70% ethanol (at  $-20^{\circ}\text{C}$ ), air-dried and resuspended in 80  $\mu\text{l}$  of ultra-pure water for use in PCR.

## Polymerase chain reaction assay

### Primers

The primers were selected on the basis of the published sequence of gI glycoprotein gene region of BHV-1 (29), which was predicted to produce a PCR product of 468 base pairs (bp) (25). The primer sequences Ext F 5'-CACGGACCTGGTGGACAAGAAG-3' position (624-645) and Ext R 5'-CTACCGTCACGTGAGTGGIACG-3' position (1070-1091) were the same as those used by Vilcek (25).

### Amplification

The PCR was performed using DNA obtained from the raw semen samples, cell culture passaged isolate and spiked semen samples. The reaction mixture contained 50 pM of each external primer, 20 mM of deoxyribonucleotide triphosphate (dNTPs), 20 mM of PCR buffer ( $\text{MgCl}_2$  50 mM), 0.75  $\mu\text{l}$  of *Taq* DNA polymerase and 5  $\mu\text{l}$  volume of sample DNA; sterile ribonuclease-free double distilled water was added to give a total volume of 50  $\mu\text{l}$ .

The PCR was performed in 35 cycles; each temperature cycle consisted of 60 s at  $95^{\circ}\text{C}$ , 60 s at  $57^{\circ}\text{C}$  and 60 s at  $72^{\circ}\text{C}$ . A final extension time of six minutes at  $72^{\circ}\text{C}$  was included at the end of last cycle.

## Analysis of polymerase chain reaction amplified products

### Electrophoresis

Ten microlitres of the PCR-amplified products of each sample were electrophoresed (85 mV for one hour) on a

1% (W/V) agarose gel in tris-borate-ethylenediamine tetra-acetic acid (TBE) in 45 mM/L tris-borate (pH 8.3) with 0.1 mM/L ethylenediamine tetra-acetic acid, and stained with ethidium bromide (0.5  $\mu\text{g}/\text{ml}$  of  $1 \times$  TBE buffer). Ready-to-use mass ruler DNA ladder, low range (MBI, Fermentas) with a size range from 80 bp to 1,031 bp were used to check the size of the amplicon. The gels were read by eye over an ultraviolet transilluminator and photographs were taken to record the results.

### Restriction enzyme analysis

The PCR-amplified product showing a clear band of 468 bp was digested with the restriction enzyme *Dde* I (Biocare, New England) to confirm the amplified product as BHV-1. Restriction fragment patterns were observed on UV transilluminator following electrophoresis on a 2% agarose gel at 10 volt/cm.

## Results

The overall prevalence of BHV-1 antibodies in breeding bulls at two farms as determined by A-B ELISA was 45.09%.

Of the 24 semen samples (12 from sero-positive and 12 from sero-negative bulls) tested, 11 samples produced distinct CPE, characterised by rounding and clumping of cells like bunches of grapes, followed by degeneration and detachment of the MDBK cell monolayer in about 72 hours to 96 hours after inoculation. The titre of the field isolates varied from  $\log_{10}^{5.24}$  to  $\log_{10}^{6.24}$  TCID<sub>50</sub>/ml. All the virus isolates were completely neutralised by BHV-1 antiserum and hence confirmed as BHV-1. The virus was isolated from 5 of the 12 sero-positive bulls (41.67%) and 6 of the 12 sero-negative bulls (50%) (Table I).

The polymerase chain reaction detected BHV-1 in 50% (6/12) and 66.67% (8/12) of the semen samples of bulls from sero-positive (Fig. 1) and sero-negative (Fig. 2) groups, respectively. In the cell culture passaged material, PCR detected BHV-1 in only those samples from which virus isolation attempts were successful (Table II).

Of 24 semen samples tested for the presence of BHV-1 by PCR and virus isolation, 17 samples were found to be positive by at least one method; 14 were PCR positive and 11 were positive by virus isolation. The sensitivity and specificity of virus isolation were 57.14% and 70% respectively, and were significantly lower than PCR. The percentages of positive and negative predictive values of virus isolation were 72.72% and 53.84%, respectively.

**Table I**  
**Detection of bovine herpesvirus-1 in sero-positive and sero-negative bulls by virus isolation and polymerase chain reaction (PCR)**

Series number	Sample designation	A-B ELISA antibody status	Virus isolation	PCR on cell culture isolate	PCR on semen
<b>Sero-negative bulls</b>					
01	L1	-	-	-	-
02	L2	-	-	-	-
03	L3	-	-	-	+
04	L4	-	+	+	-
05	L5	-	-	-	+
06	L6	-	+	+	+
07	L7	-	+	+	+
08	L8	-	-	-	-
09	L9	-	-	-	+
10	L10	-	+	+	+
11	L11	-	+	+	+
12	L12	-	+	+	+
<b>Sero-positive bulls</b>					
13	P1	+	-	-	-
14	P2	+	-	-	-
15	P3	+	-	-	-
16	P4	+	-	-	+
17	P5	+	+	+	-
18	P6	+	+	+	-
19	P7	+	+	+	+
20	P8	+	+	+	+
21	P9	+	-	-	-
22	P10	+	-	-	+
23	P11	+	-	-	+
24	P12	+	+	+	+
<b>Total</b>	<b>24</b>		<b>11</b>	<b>11</b>	<b>14</b>

The L and P series bulls originated from two different bull stations located about 90 km apart  
 A-B ELISA: avidin-biotin enzyme-linked immunosorbent assay

**Table II**  
**Detection of bovine herpesvirus-1 infection by virus isolation and polymerase chain reaction (PCR)**

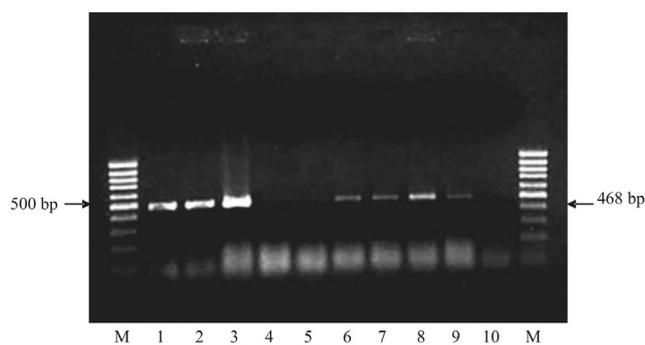
Series number	Test employed	Number tested	Number positive	Percentage positive
<b>Sero-positive bulls</b>				
1	Virus isolation and VNT	12	5	41.66
2	PCR on semen	12	6	50
3	PCR on cell culture isolates	12	5	41.66
<b>Sero-negative bulls</b>				
1	Virus isolation and VNT	12	6	50
2	PCR on semen	12	8	66.66
3	PCR on cell culture isolates	12	6	50

VNT: virus neutralisation test

## Discussion

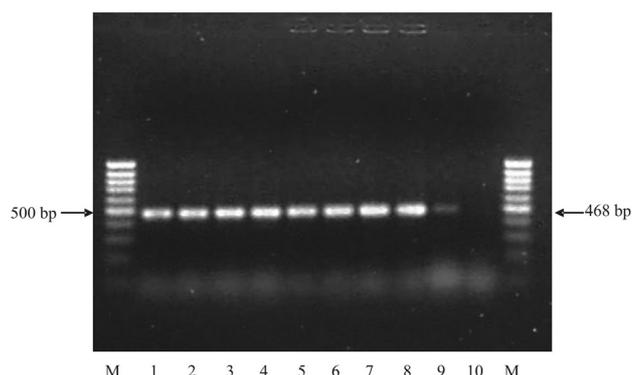
The conventional methods for detection of BHV-1, such as virus isolation, the fluorescent antibody test, ELISA and serological testing, lack sensitivity, particularly when

applied to semen donor bulls (33). Although there are arguments for ensuring that bulls at AI centres are BHV-1 sero-negative, the risk of transmission of the virus from such bulls cannot be eliminated. However, the threat of transmission of the virus through semen can be reduced or eliminated by adopting vigilant hygienic and regulatory



Lane M deoxyribonucleic acid molecular weight marker (80 bp-1,031 bp)  
 Lane 1 standard bovine herpesvirus-1  
 Lane 2 P4  
 Lane 3 P7  
 Lane 4 P5  
 Lane 5 P6  
 Lane 6 P8  
 Lane 7 P10  
 Lane 8 P11  
 Lane 9 P12  
 Lane 10 blank control

**Fig. 1**  
**Polymerase chain reaction amplification of bovine herpesvirus-1 from semen samples of sero-positive bulls**



Lane M deoxyribonucleic acid molecular weight marker (80 bp-1,031 bp)  
 Lane 1 standard bovine herpesvirus-1  
 Lane 2 L3  
 Lane 3 L5  
 Lane 4 L6  
 Lane 5 L7  
 Lane 6 L9  
 Lane 7 L10  
 Lane 8 L11  
 Lane 9 L12  
 Lane 10 blank control

**Fig. 2**  
**Polymerase chain reaction amplification of bovine herpesvirus-1 from semen samples of sero-negative bulls**

measures supported by routine serological and virus detection tests. The present study aimed to evaluate different laboratory procedures for effective diagnosis of BHV-1 infection in breeding bulls.

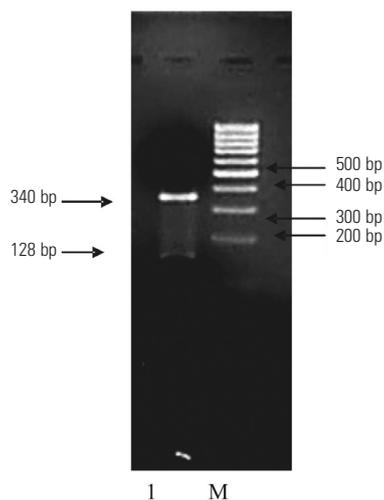
The study has shown a 45.09% overall prevalence of BHV-1 antibody in apparently healthy breeding bulls, as shown by A-B ELISA. Several research workers have reported a high to moderate prevalence of antibodies to BHV-1 in India. Renukaradhya *et al.* (20) have reported prevalences of 95% and 41% in breeding bulls over Tamil Nadu and Karnataka, respectively. Singh *et al.* (23) found 32.34% bulls positive for BHV-1 at a bull farm in Punjab, India. The seropositivity rate of Jerseys (75%) and Holstein Friesians (84.6%) is higher than that of crossbred (37.5%) bulls. This difference may be attributed to their exotic germplasm, which renders them more susceptible to diseases and environmental stress factors. Conversely, the lower prevalence rate in crossbred bulls might be due to their relatively high resistance to diseases and better adaptation to the environmental conditions. No significant differences in positivity rate were observed in diverse age groups.

A distinct CPE characteristic of herpesvirus was observed on second passage onward on virus isolation from the semen samples. For any isolate to be considered to be BHV-1, its neutralisation index should be greater than 1.5 (15). In this study, 11 isolates (45.83%) were considered to be completely neutralised by standard BHV-1 antiserum as their neutralisation indexes were more than 1.5 (Table I). The neutralisation test has been consistently used for the confirmation of BHV-1 isolates by many diagnosticians (10, 13, 14). Weiblen *et al.* (28) reported isolation of BHV-1 from 9 out of 11 preputial swabs and 2 out of 11 semen samples. Oirschot *et al.* (16) reported isolation of the virus from semen samples of 43 out of 116 apparently healthy bulls.

The split-sample method was used for PCR in order to control false negative results. A duplicate of each semen sample was spiked with an aliquot of known BHV-1 (titre log 10<sup>6</sup> TCID<sub>50</sub>/0.1 ml) and tested in parallel with the control sample. The sample was considered negative if the non-spiked sample and spiked sample showed negative and positive results, respectively. If both spiked and non-spiked samples showed positive then the sample was considered positive. The DNA extracted from the semen

samples as well as cell culture passaged isolates of both sero-positive and sero-negative bulls showed a very clear, 468 bp band upon amplification. Specific cleavage of the PCR products by a restriction enzyme, *Dde* I, yielded fragments of predictable sizes – 340 bp and 128 bp – indicating specific amplification of the BHV-1 genome (Fig. 3). Similar results have been reported by *Vilcek et al.* (26) and *Rocha et al.* (21).

The polymerase chain reaction detected BHV-1 directly from semen samples of 58.33% (14 out of 24) bulls and 45.83% (11 out of 24) cell culture passage isolates. The PCR could not amplify viral DNA from three samples extracted directly from semen, whereas the samples were found to be positive when PCR was done on the cell culture passaged isolates. The failure to amplify the DNA in these three samples may be due to the inhibitory effect of the bovine semen on PCR. On the other hand, six samples which were negative for virus isolation were found positive in a PCR done directly from semen samples. This may be because virus isolation from semen in cell culture normally requires a dilution of the semen to eliminate cytotoxicity or inhibitory factors (31), which further reduces the sensitivity of the virus isolation. Semen samples that had only a very minute quantity of BHV-1, might have given a negative result when further diluted (1:10 in growth medium) to avoid cytotoxicity in cell culture isolation of the virus.



Lane M deoxyribonucleic acid molecular weight marker (80 bp-1,031 bp)  
Lane 1 *Dde* I digest of standard bovine herpesvirus-1 DNA

**Fig. 3**  
***Dde* I digest of polymerase chain reaction amplified product of bovine herpesvirus-1**

Polymerase chain reaction has been used for detection of BHV-1 in bovine semen by various researchers (11, 21, 24). *Engelenburg et al.* (7) developed a PCR assay to detect BHV-1 in bovine semen using a purification method that eliminates interfering components. *Yason et al.* (32) used a gene releaser to extract DNA and found that the sample prepared by the gene releaser showed a 100-fold increase in sensitivity compared with standard DNA extraction and modified proteinase K digestion.

For PCRs to be used in routine screening of semen, the importance of a PCR-positive, virus-isolation-negative sample is yet to be established. Our results clearly showed that PCR is an invaluable tool for rapid detection of BHV-1, particularly in semen samples when the inhibitory factors have been removed.

The comparative results of the two BHV-1 detection techniques – virus isolation and PCR – are presented in Table II. As described above, the overall prevalence of BHV-1 infection as detected by virus isolation and PCR was 45.83% and 58.33%, respectively. Virus isolation technique could identify 41.67% (5 out of 12) and 50% (6 of 12) of samples as positive, while PCR in semen could detect 50% (6 out of 12) and 66.67% (8 out of 12) of samples as positive from sero-positive and sero-negative bulls, respectively. The sero-negative bulls in which IBR virus was detected by PCR or virus isolation had previously been infected by contact with other co-located sero-positive cattle, and had probably been sero-positive for some time. The virus could be detected in fewer samples (11 out of 24) by PCR done on cell culture isolates than those directly from neat semen samples (14 out of 24). This may be due to the failure of virus isolation in MDBK cell line from a few samples because of the very low concentration of virus in the initial inoculum, which was further reduced by dilution of the samples (1:10 in growth medium).

All the virus isolation positive samples were also shown as positive by PCR done on cell culture isolates, indicating that PCR is a more sensitive technique than virus isolation in detecting BHV-1 infection in breeding bulls. *Engelenburg et al.* (8) showed that PCR could detect five times more positive semen samples for BHV-1 than the virus isolation. *Xia et al.* (31) reported that PCR with Southern blot hybridisation was the most sensitive method and could detect BHV-1 in semen of artificially infected bulls for a longer period than virus isolation. *Wagter et al.* (27) detected BHV-1 DNA in 73 ejaculates out of 162, whereas virus isolation could detect only 51 ejaculates as BHV-1 positive.

There was not always a correlation between antibody status and virus excretion in semen, since both BHV-1 recovery by virus isolation and BHV-1 DNA detection by PCR were

also obtained from the sero-negative bulls (Table II). Other workers have also reported detecting BHV-1 in samples provided from sero-negative bulls (17). Rocha *et al.* (21) reported BHV-1 recovery by virus isolation and BHV-1 DNA detection by PCR from sero-negative bulls.

To certify the absence of IBR virus in sero-negative bulls present alongside sero-positive cattle on the same farm, a PCR can be performed on semen or nasal discharges. However, the results cannot certify the absolute absence of IBR virus particles, which may be present at a level under the detection limit of PCR. The recommendation for international or even national trade in semen from genetically selected bulls is that an eligible bull should be born on a farm that is certified as having been IBR free for more than two years and that all cattle on the farm, including the bulls, should have been sero-negative throughout that period.

A marked difference was observed between the proportion of PCR-positive and virus isolation-negative samples. Six virus isolation-negative samples were found positive by

PCR, indicating that PCR is more sensitive than virus isolation in detecting BHV-1 from semen samples.

## Conclusions

The seroprevalence of BHV-1 infection in apparently healthy breeding bulls was found to be 45.09%. Polymerase chain reaction tests to detect bovine herpesvirus-1 in semen were found to be more sensitive than virus isolation methods. Since the virus could be detected by virus isolation and PCR in sero-negative bulls, therefore, the fact that bulls have a sero-negative status does not eliminate the risk of virus transmission through semen. Polymerase chain reaction with suitable modification to eliminate the inhibitory factors present in the semen can replace virus isolation for quick and reliable diagnosis of BHV-1. ■

## Détection de l'infection par l'herpèsvirus-1 bovin dans la semence de taureaux reproducteurs par isolement viral et amplification en chaîne par polymérase

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### Résumé

Une épreuve immuno-enzymatique à l'avidine-biotine a été utilisée pour rechercher la présence d'anticorps dirigés contre l'herpèsvirus-1 bovin (BHV-1) dans des échantillons de sérum prélevés chez 51 taureaux reproducteurs apparemment sains. Elle a mis en évidence un taux de prévalence des réactions positives de 45,09 %. Des échantillons de semence ont ensuite été recueillis chez 12 des taureaux à sérologie positive et chez 12 des taureaux à sérologie négative. Ces échantillons ont été soumis à la fois à une épreuve d'isolement du virus et à une amplification en chaîne par polymérase (PCR) pour rechercher l'antigène BHV-1. L'épreuve PCR était appliquée pour détecter l'acide désoxyribonucléique du BHV-1 à l'aide d'amorces sélectionnées à partir de la séquence relativement conservée du gène codant pour la glycoprotéine g1 pour amplifier un fragment contenant 468 paires de bases. Il a été confirmé que les produits amplifiés par la PCR correspondaient au BHV-1 grâce à l'utilisation d'une enzyme de restriction, *Dde* 1, qui a produit des fragments de taille prévisible, à savoir de 340 et de 128 paires de bases. Les résultats positifs à l'épreuve d'isolement du virus, confirmés par neutralisation virale, ont mis en évidence la présence de l'antigène BHV-1 dans la semence de six taureaux à sérologie positive et de cinq taureaux à sérologie négative. Par comparaison, les résultats positifs à l'épreuve PCR correspondaient à la découverte du génome

du BHV-1 dans la semence de six taureaux à sérologie positive et de huit taureaux à sérologie négative. Sur les 24 échantillons de semence testés, le nombre d'échantillons ayant donné des résultats positifs était de 14 avec le PCR et de 11 avec l'isolement viral. Comparativement à l'épreuve PCR, l'isolement du virus était associé à une sensibilité de 57,14 % et à une spécificité de 70 %. Dans les échantillons de semence prélevés chez des taureaux à sérologie négative, le BHV-1 a été détecté plus souvent par les méthodes d'amplification en chaîne par polymérase que par isolement du virus, ce qui indique que la PCR est plus sensible pour le dépistage du BHV-1 chez les taureaux.

#### **Mots-clés**

Amplification en chaîne par polymérase – Herpèsvirus-1 bovin – Isolement viral – Semence – Taureau reproducteur.



## **Detección de infección por herpesvirus bovino 1 en semen de toros reproductores mediante el aislamiento del virus y la técnica de reacción en cadena de la polimerasa**

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#### **Resumen**

Los autores examinaron muestras de suero de 51 toros reproductores, aparentemente en buen estado de salud, para detectar la presencia de anticuerpos del herpesvirus bovino 1 (HVB-1) mediante la técnica de inmunoabsorción enzimática con avidina-biotina, que arrojó una prevalencia de reacciones seropositivas del 45,09%. Posteriormente se recogieron muestras de semen de 12 toros seropositivos y 12 seronegativos. Se les hizo una prueba de aislamiento viral y una de reacción en cadena de la polimerasa (PCR) para buscar el antígeno del HVB-1. La técnica PCR se empleó para detectar el ácido desoxirribonucleico del HVB-1 mediante la utilización de cebadores escogidos en la secuencia relativamente preservada del gen de la glucoproteína g1 para amplificar un fragmento de 468 pares de bases. Se confirmó, mediante la enzima de restricción *Dde* 1, que los productos amplificados por PCR correspondían al HVB-1 dado que produjo fragmentos de longitudes previsibles, a saber, de 340 y 128 pares de bases. Los resultados positivos de la prueba de aislamiento viral, confirmados por neutralización viral, demostraron la presencia del antígeno del HVB-1 en el semen de seis toros seropositivos y cinco seronegativos. En comparación, los resultados positivos obtenidos con la prueba PCR demostraron la presencia del genoma del HVB-1 en el semen de seis toros seropositivos y ocho seronegativos. De las 24 muestras de semen analizadas, 14 dieron resultados positivos con la prueba PCR y 11 con el aislamiento viral. En comparación con la prueba PCR, la sensibilidad y especificidad del aislamiento viral fueron del 57,14% y el 70% respectivamente. En las muestras de semen pertenecientes a toros seronegativos, el HVB-1 se detectó con mayor frecuencia con las pruebas de PCR que con las de aislamiento viral, por lo cual se deduce que el método PCR es más sensible para la detección del HVB-1 en toros.

#### **Palabras clave**

Aislamiento viral – Herpesvirus bovino 1 – Reacción en cadena de la polimerasa – Semen – Toro reproductor.



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