

Bovine herpes virus-1 (BoHV-1) detection in dairy cattle with reproductive problems in Sudan

Amira Mohamed Elhassan^{1,*}, Mohamed Abdalla Fadol², Mohamed Ahmed Mohamed Salih³, Abdel Rahim Mohamed El Hussein¹

¹Virology Department, Veterinary Research Institute, Khartoum, Sudan;

²Viral Vaccine Production, Veterinary Research Institute, Khartoum, Sudan;

³Molecular Biology Department, Central Laboratory, Khartoum, Sudan.

*Corresponding author's e-mail: amirahassan98@hotmail.com

ABSTRACT

The present work aimed to observe the infection pattern of Bovine herpes virus-1 (BoHV-1) in dairy cattle with reproductive problems in Sudan. A total of 140 samples comprising of vaginal swab (n=97), placenta (n=15), whole blood (n=19), uterine fluid (n=1), and serum (n=8) were collected from 16 dairy herds showing particularly high rate of abortion and infertility in Khartoum State. The samples were used for virus isolation, and were tested by Enzyme-Linked Immunosorbent Assay (ELISA) and polymerase chain reaction (PCR). No virus could be isolated from the samples inoculated for isolation in cell culture. Out of 80 specimens tested by ELISA, 7 (8.75%) were found to be positive, and one sample was doubtful. Using PCR, 11 (10.7%) out of 103 samples were found to be positive. When comparing between two methods for DNA extraction, the DNA extracted by commercial kit was found to be better in quality as compared to the DNA extracted using phenol/chloroform/isoamyl-alcohol method. The study confirmed the presence of BoHV-1 in cattle farms with reproductive problems in Sudan.

Keywords

BoHV-1, Dairy cattle, Reproductive problems, Sudan

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INTRODUCTION

Bovine herpesvirus-1 (BoHV-1) may cause severe respiratory disease, venereal disease with reduced reproductive performance, and abortion (Muylkens et al., 2007). Genital infection caused by BoHV-1 subtype 2 may occur in both males and females. In cows and heifers, acute infectious pustular vulvovaginitis (IPV) develops within 1-3 days of mating or close-contact with infected animal even if there are no visible lesions. The transmission may also occur during artificial insemination with infected semen from subclinically infected bulls (Allen et al., 2005).

Abortion caused by BoHV-1 can be tentatively diagnosed by identifying the characteristic clinical signs and lesions. The virus can be demonstrated in fetal tissues, placenta, cotyledons, amniotic fluid, vaginal swabs and samples from the dam uterus (Allen et al., 2005).

There are two ways to diagnose BoHV-1 viz., direct identification of the agent or its components or indirectly by detecting the specific antibody response in serum samples. Enzyme-Linked Immunosorbent Assay (ELISA) was developed for the detection of BoHV-1 antigen in nasal swab specimens collected from the infected animals (Collins et al., 1985). ELISA is considered to be highly useful for the diagnosis of BoHV-1 infection. Furthermore, the antigen capture ELISA showed good sensitivity (64%) and specificity (100%) when the samples were tested for the presence of BoHV-1 antigen (Collins et al., 1985). PCR assay has been recorded as a good alternative method for virus detection since the results of the PCR assay can be

found within short time whereas the virus isolation and subsequent identification method needs longer time (Van Engelenburg et al., 1993).

In Sudan, BoHV-1 isolation was first reported in 1983, and other researches revealed the existence of the disease which was confusing clinically with rinderpest (Eisa, 1983; Hassan et al., 1985). El Hussein et al. (2005) reported high prevalence (60%) of BoHV-1 antibodies in Khartoum State. In sero-epidemiological surveys using ELISA in cattle revealed the existence of antibodies against BoHV-1 in 13 States with prevalence ranging from 16.67% in West Kordofan to 68% in River Nile States (Elhassan et al., 2006). Elhassan et al. (2011) recorded a survey to determine prevalence of antibodies against BoHV-1 in dairy cattle in farms with reproductive problems in two areas in Sudan. The disease showed higher prevalence in Khartoum state (51.7%) while in central Sudan, the prevalence was lower (32.7%). This study aimed to investigate BoHV-1 in dairy cattle suffering from reproductive problems in Sudan. The study also aimed to evaluate the usefulness of methods for BoHV-1 investigations by means of virus isolation, ELISA and PCR.

MATERIALS AND METHODS

Samples: Dairy cattle herds with reproductive problems including infertility and abortion in Khartoum State were identified and selected using a questionnaire survey that was done with the dairy farms in the area prior to collection of samples. A total of 140 samples were collected from 16 dairy herds with complains of reproductive problems around Khartoum State. The major complains included infertility (81.3%), abortions at different stages of pregnancy (68.8%), neonatal death (death after 1-2 days after birth) (37.5%), stillbirth (18.8%) and congenital defects (6.3%). The specimens showing clinical signs were collected from all cases. The collected samples included whole blood (n=19), placenta (n=15), vaginal swabs (n=97), serum (n=8), and uterine fluid (n=1) (Table I). Only a single sample was obtained from each animal as judged appropriate at the time of sampling.

Detection of viral antigen using cell culture

Reference BoHV-1 virus: A reference BoHV-1 (IBR.M/KH.BK.P2) used as control virus was obtained from Agricultural Research Institute, Kenya. It was propagated twice in bovine kidney cells (BKC), lyophilized and kept at -20°C at the Virology Department, Veterinary Research Institute.

Virus isolation

Samples preparations: The vaginal swabs were vigorously rubbed against the mucosal surfaces and immediately immersed in tubes with 2 mL transport medium, and were transported on ice to the laboratory where they were left to soak overnight at 4°C and stored at -20°C until examination. Whole blood samples were prepared by washing the blood 3 times in phosphate buffer solution (PBS) using cold centrifuge, then resuspended as 10% in PBS. Ten percent homogenates (W/V) from suspected organs and placenta were prepared in PBS containing 200,000 IU Pencillin, 100 mg Streptomycin sulphate and 50 units Mycostatin/mL using a homogenizer (Kinematic Aglittau-Switzerland). The homogenate suspension was centrifuged at 3,000 rpm for 10 min. Then the supernatant was collected and kept at -20°C until used. All the samples were prepared and inoculated for virus isolation in BKC culture.

ELISA for detection of BoHV-1 virus antigen: Sandwich ELISA for detection of BoHV-1 (Bio X Diagnostics, Belgium) was used to test 80 Samples (Table I) as recommended by the manufacturer. The optical density was read using plate reader (Anthos 2020, UK) and 450 nm filter, limit of OD positivity for the antigen was equal to or greater than 0.150. The test was validated only if the positive serum yielded a difference in optical density that was greater for each case than the values given on the quality control (QC) data sheet.

Polymerase chain reaction (PCR)

Purification of virus & samples: Reference control and samples of suspected BoHV-1 cell culture harvest, swabs and tissue homogenates suspensions were centrifuged at 3,000 rpm for 15 min and the supernatant was used for DNA extraction. BoHV-1 DNA extraction and purification were done using two different methods: a) phenol/chloroform/isoamyl-alcohol DNA extraction was done according to Rola et al. (2005); b) DNA was extracted using commercially available Kit (Qiagen, Germany), as per the manufacturer's protocol. The DNA quantity and quality were measured using nano-drop 1000 spectrophotometer V3.7 (Thermo Fisher Scientific, USA).

The primer sequences were designed based on the sequence of the BoHV-1 glycoprotein E (gE) gene. The sequence of the oligonucleotides were gE-1 5'-GCTTCGGTTCGACACGGTCTT-3' and gE-2 5'-CTTGTGCGCCCGTTGAGTTCG-3'. Amplicons were

visualized under ultraviolet (UV) light after electrophoresis of 10 μ L of the PCR products in a 2% agarose stained with ethidium bromide (0.3 mg/mL) using 100-bp molecular weight marker (Vivantis, Malaysia).

RESULTS AND DISCUSSION

Many causes of reproductive disorder such as abortion, infertility, neonatal death, stillbirth and congenital defects remain unknown. However, infectious agents including virus, bacteria, parasite and fungus are known as important agents in causing these problems. These disorders adversely affect the reproductive efficiency and reproductive longevity of female livestock (De-Vecchio et al., 1992).

During the course of this study, 140 specimens collected from clinically suspected animals were screened for the presence of viral agents. None of the tested samples proved positive for virus isolation (Table 1). The failure to isolate BoHV-1 in cell culture might be due to the need of virus particle to be infective, BoHV-1 usually cause autolysis of the fetus that is rarely expelled in a fresh state for virions (Kahrs, 2001). On the other hand, the virus amount in peripheral blood could be very low to be detected by the procedure used in this study; this was in agreement with the findings of Van Der Maaten and Meller (1985).

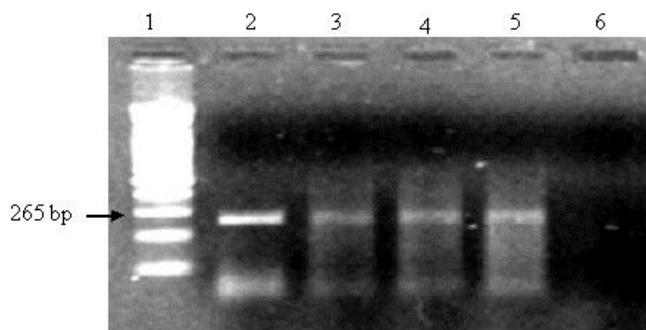


Figure 1. Agarose gel electrophoresis for the PCR amplicons of the glycoprotein E gene of bovine herpes virus-1 (BoHV-1). lane 1- DNA ladder (100 bp); lane 2- BoHV-1 positive control; lanes 3, 4 and 5- bovine specimens; lane 6- negative control.

When using the ELISA method, 7 samples (8.75%) out of 80 specimens tested were found positive while one sample (1.25%) was doubtful (Figure 1, Table 1). However; using PCR, 11 samples (10.6%) out of 104 samples tested were positive. This finding was in agreement with the conclusion that the sensitivity and specificity of PCR are generally higher than virus

isolation or capture ELISA procedure (Office International des Epizootés (OIE, 2000). It is worth noting that all PCR positive samples were vaginal swap samples indicating that these samples might be most appropriate for this technique. On the other hand, viral antigens were mostly detected from placenta where virus antigens might be abundant (Table 2). However, both Ag ELISA and PCR showed high agreement (87.7%) in detecting BoHV-1 in the 80 samples that were tested using both techniques. Only one sample was positive while 63 samples were negative (Table 3).

During the course of this investigation and in order to establish a method for DNA extraction; two methods were assessed using a commercially available DNA extraction kit and the phenol/chloroform/isoamyl-alcohol method. The quality and quantity of DNA appeared to be good when using Qiagen commercial kit. However, the phenol/chloroform/isoamyl-alcohol extraction method was also useful and could be effective when dealing with more hazardous organisms.

The results of the present laboratory testing for viral antigens confirmed BoHV-1 to be one of the causative agents of reproductive diseases observed in the dairy cattle herds in Sudan. This strongly corroborated the findings of Elhassan et al. (2006) who revealed high seroprevalence of BoHV-1 in dairy cattle with reproductive disorders in Khartoum State and Central Sudan (51.7% and 32.7 %, respectively). These authors stated that BoHV-1 antibodies were highly prevalent in dams suffering from abortion and infertility.

The results of the present investigation indicated that the PCR was a good alternative for the rapid detection of the BoHV-1 in vaginal swaps. However, BoHV-1 antigen detection using ELISA was beneficial in testing other types of samples (Table 3). Nonetheless, although PCR could be used as the first choice for routine diagnosis of BoHV-1, the most reliable result would always be derived from combining PCR analysis with other techniques, such as virus isolation, ELISA and immunofluorescence (Van Engelenburg et al., 1993). This is supported by the results of present study that indicated that both ELISA and PCR techniques were useful for diagnosis of suspected BoHV-1 infections in situations where virus isolation was not possible.

Health certification might be required for a variety of commercial purposes. These include screening of bulls for entry to artificial insemination (AI) centers, selection of both donors and recipient cows for embryo transfer, and the sale and introduction of animals to the

Table 1. Results of positive samples of bovine specimens collected from cases with reproductive problem in Sudan tested by ELISA, virus isolation and PCR.

Test	Samples: N o. positive/No. tested (%)					Total
	V.S.	Pla	W.B.	U.F.	S	
Ag ELISA	1/59 (1.7)	5/15 (33.3)	1/5 (20)	0/1 (0)	-	7/80 (8.8)
Virus Isolation	0/97 (0)	0/15 (0)	0/19 (0)	0/1 (0)	0/8 (0)	0/140 (0)
PCR	11/92 (11.95)	0/5 (0)	0/5 (0)	0/1 (0)	0/1 (0)	11/104 (10.6)

V.S.=vaginal swab, Pla= Placenta, W.B.= Whole blood, U.F. = Uterine fluid, S = Serum Ag ELISA= Antigen detection ELISA.

Table 2. Results of the purity and concentration of DNA extracted by phenol/ chloroform/ isoamyl-alcohol and kit extraction

Method	Substance	Reading ng/ μ L (Average)	Purity (Average)
phenol/chloroform/ isoamyl-alcohol	Reference virus	23.7	1.36
	Samples	1.7 - 28.5 (11.2)	1.16 - 1.8 (1.3)
Kit extraction	Reference virus	12.3	1.55
	Samples	1.4 - 46.3 (19.1)	1.38 - 4.7 (1.8)

Table 3. Comparison between PCR and ELISA in detection of BoHV-1

	ELISA		Total
	Positive	Negative	
PCR	Positive	10	11
	Negative	63	69
	Total	73	80

herds of known disease status. It is usual to test animals first to detect specific antibodies to BoHV-1 as an indication of past and probable latent infection with BoHV-1. For many purposes, the detection of antibodies to BoHV-1 will render the animal ineligible for the purpose for which it was being tested. The use of semen from an IBR serologically positive and antigen negative animal is admitted and prescribed by the OIE (Terrestrial Animal Health, 2012).

CONCLUSION

In view of the expanding genetic upgrading programs through artificial insemination, continuous investigations on a large number of samples from different regions are necessary to allow the description and evolution of circulating strains of BoHV-1 in

Sudan. Both Ag detection ELISA and PCR are useful in BoHV-1 diagnosis of suspected IBR infection in samples collected under field conditions in Sudan.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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