



Epidemiology of bovine herpes virus-1 under different housing practices in cattle breeding stations

B M CHANDRANA¹, D RATHNAMMA², S S PATIL³, S RANGANATHA⁴, RAMESH C KOVI⁵, D S AKHILA⁶, SHRIKRISHNA ISLOOR⁷, C RENUKAPRASAD⁸ and K PRABHUDAS⁹

Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka 585 401 India

Received: 18 September 2013; Accepted: 18 October 2013

ABSTRACT

Present paper describes the dynamics of bovine herpes virus-1 (BoHV-1) in breeding cattle under different housing, feeding and watering practices. Organized breeding farms A, B, C, D were selected for this study. In farm A, the animals were housed in large open shed with common grazing and drinking area. Farm B had individual pens with separate feeding facility but with common watering/drinking facility. Farm C had had individual pens, with separate feeding and drinking facility for each animal. Farm D possessed modern individual housing system with separate feeding, drinking facility, restricted personnel entry and better bio-security set up. The blood and semen samples / vaginal swabs were collected from 177 animals. Avidin- biotin ELISA recorded BoHV-1 antibodies in 56, 38.77, 21.05 and 17.5% animals in farm A, B, C and D respectively. A TaqMan probe real time PCR targeting the BoHV-1 gB gene was standardised and this assay detected BoHV-1 in 11, 3 and 3 animals in Farm A, B and C respectively. None of the samples collected from Farm D were positive for BoHV-1 by real time PCR. The study recorded higher seroprevalence as well as virus transmission in farms that had housing systems allowing closer animal to animal contacts. In view of the different modes adopted by BoHV-1 in transmission through susceptible populations, the study recommends better bio-secured housing systems that avoid closer animal to animal contacts, for production of BoHV-1 free semen and calves at breeding stations.

Key words: Epidemiology, Bovine herpes virus-1, Housing practices

Bovine herpes virus (BoHV-1), a member of the family *Herpesviridae*, causes diverse ailments, viz. infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), infectious balanoposthitis, keratoconjunctivitis and neurological disorders (Patil *et al.* 2012). Though mucous membranes of either upper respiratory or genital tract are the most common routes of entry of BoHV-1, transmission by conjunctival and aerosol routes were also reported (Leuzinger *et al.* 2005). During primary infection, the virus is transported along axons where it becomes latent and persists for life in sacral and trigeminal ganglions. The virus

gets reactivated under stress conditions making the animal a potential shedder of virus (Chintu *et al.* 2012). Hage *et al.* (1996) in their study on population dynamics of BoHV-1 defined the reproduction ratio of BoHV-1 to be at a minimum of 7. This study described the virus transmission and seroprevalence of BoHV-1 in breeding cattle, under different housing, feeding and watering practices.

MATERIALS AND METHODS

Selection of farms and sample collection: To study the epidemiology of bovine herpes virus -1 under different housing conditions, 4 organized breeding farms (A, B, C and D) were selected that followed varied management practices in respect to housing, feeding and watering of animals.

Farm A having only indigenous breeding animals, housed bulls in individual pens with common feeding and drinking facility for all animals. The females were let loose with common housing in large open animal shed with common grazing lands, drinking tanks with close social relationships among animals. Semen samples from 2 breeding bulls, vaginal swabs from 48 cows and blood samples from all these 50 (2 bulls and 48 cows) animals were collected at Farm A.

Present address: ¹Scientist-2, IAH and VB (dinglychandru@yahoo.com), ³Senior Scientist, PD-ADMAS (sharnaspin123@rediffmail.com), ⁹Retired Project Director (prabhudask@gmail.com), PD-ADMAS, Hebbal, Bengaluru, Karnataka. ²Professor and Head (doddamanerathnamma@yahoo.co.in), ⁴M.VSc. Scholar (srangavetco@gmail.com), ⁶Research Associate (akhilads87@gmail.com), ⁷Associate Professor, Dept of Veterinary Microbiology (kisloor @rediffmail.com), ⁸Vice Chancellor (c.renukaprasad@gmail.com), ⁵Assistant Professor, Department of Veterinary Population Medicine, University of Minnesota, St. Paul, Minnesota, USA (rckovi@gmail.com).

Farm B had HF cross and Jersey crossbreeding bulls. The bulls were housed in individual bull pens, with separate feeding facility for each animal. The drinking facility was in between 2 bull pens and this facility was common for bulls placed in 2 adjacent pens. Semen and blood samples were collected from 49 breeding bulls stationed at Farm B.

Farm C had only HF cross female cattle. The farm had individual pens type of housing, with separate feeding and drinking facility for each animal. Vaginal swabs and blood samples were collected from 38 cows at Farm C.

Farm D had only Murrah breeding buffalo bulls. The farm possessed modern individual pen type of housing, with separate feeding and drinking facility for each animal. The entry of personnel was restricted and was limited to only the regular animal handlers. All the entry and exit inside the animal premise was digitally monitored with high hygienic practices. Semen and blood samples were collected from 40 breeding bulls at Farm D.

Virginal swabs and semen samples were collected in viral transport media and were transported to the laboratory under cold chain and stored at -80°C until further use.

The sample collection at all the farms was made exclusively during early mornings, which recorded sudden change of weather conditions such as sudden rains and adverse cold weather.

Enzyme linked immunosorbent assay: ELISA was used to study serum samples collected from all the animals. The avidin-biotin ELISA kits were used for this purpose; and 1:30,000 dilution of biotin anti IgG conjugate and 1: 15,000 dilution of avidin-HRPO conjugate were used in ELISA.

TaqMan real time PCR

Extraction of DNA: The method for extraction of DNA from semen was essentially according OIE protocols. The DNA from cell culture supernatants and swabs samples collected from animals was isolated as per Lopasev *et al.* (1991).

Primers and probe sequences: A new set of primers and TaqMan 5'-nuclease probe was designed, targeting a 71 bp conserved region on the glycoprotein B gene (gB) of the virus. The primers and probe were checked for their specificity using the database similarity search programme nucleotide-nucleotide BLAST (www.ncbi.nlm.nih.gov/blast) and the sequences were 100% homologous to those of BoHV-1 strains deposited in the GenBank. The designed TaqMan probe was a sequence-specific oligonucleotide labelled with 2 different fluorophores, the reporter/donor, 5-carboxyfluorescein (FAM) at the 5' end, and the acceptor/quencher 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. This real-time PCR assay was designed to detect viral DNA of all BoHV-1 strains, including subtype 1 and 2, from bovine semen as well as from clinical samples. The sequences of the designed the primers and probes were ; Primer gB-F: 5'-TGCTCGACTACAGCGAGATACAG-3' ; Primer gB-R:

5'-CACGCGGTCAATGTCGTAGA3' and the TaqMan probe: 5'-FAM CCGCAACCAGCTGCACGAGCTAMRA-3'.

The real-time PCR was carried out using the platinum quantitative PCR super mix-UDG master mix. A number of experiments were performed to optimize the PCR protocol, including concentration of reagents and PCR cycling parameters. The optimized PCR assay was established using 25 μL . Non templates control (NTC, reagents only) and negative controls were included in each test. The optimised 25 μL of reaction mixture containing 12.5 μL of 2X PCR Super mix, 1 μL each of forward primer and reverse primer containing 10 pmoles/ μL , 1 μL of TaqMan probe containing 5 pmoles/ μL and 4.5 μL nuclease free water. 5 μL of the DNA extracted from cell culture supernatants/ clinical swab/ semen sample was added to the PCR reagent mixture to make final volume of 25 μL . PD_ADMA5 BoHV-1 isolate was used as positive control during the process of real time PCR standardization.

The real time PCR plates loaded with reaction mixture were placed in the real-time PCR detection system with the standard cycling parameters of one cycle 95°C for 10 min followed by 40 repeated cycles of 95°C for 15 sec and 60°C for 45 sec.

The results were analyzed using SDS 2.1 version of software provided with 7900 ABI Real time PCR system. The standardization of real time PCR was carried out at National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru, India.

RESULTS AND DISCUSSION

One of the important characteristics of BoHV-1 is establishment of life long latency in sensory neurons of the peripheral nervous system after replication in mucosal epithelium. Bovine herpes virus-1 is thought to penetrate the terminus of the sensitive nerves distributed in the infected epithelium and transported along the microtubules of the axons to reach the neuron body in the nervous ganglion. During latency, a latency related transcript (LRT) region is expressed in BoHV-1 leading to the inhibition of the lytic cycle and the induction of an anti-apoptotic state of the infected cells (Ranganatha *et al.* 2013).

Mucous membrane of either upper respiratory or genital tract is the most common route of entry of BoHV-1. Transmission by conjunctival route was also reported. Direct nose to nose contact is the preferential way of transmission of BoHV-1 (Benoit *et al.* 2007). However, air-borne transmissions by the aerosol route were demonstrated on short distances (Leuzinger *et al.* 2005). Genital infection requires direct contact at mating. Genital transmission also occurs through virus contaminated semen. Hence, the associations between animals in a herd/farm appear to be a key factor in spread of the virus. Keeping this key epidemiological factor in view, we collected samples from

177 breeding animals from 4 organized breeding farms (Table 1), having different managerial practices like feeding, watering and housing to evaluate the relative disease dynamics under varied managerial practices in similar environmental conditions.

The sample collection was made exclusively in the early mornings which recorded sudden change of weather conditions like sudden rains and adverse cold weather conditions. It was demonstrated that the corticosteroids released naturally (due to stress) or administered intentionally cause reactivation of latent BoHV-1 virus in carrier animals (Hage *et al.* 1996). In animals, sudden change of weather is a major stress factor, which may reactivate the latent virus into shedding. The selection of season for sample collection during our study was in accordance with Patil *et al.* (2012) who found that intense winter as the most suitable season for collection of clinical samples from BoHV-1 carrier animals for virus detection.

During our sample collections, though most of the breeding animals appeared healthy, a countable number of them had lesions of balanoposthitis, pustular vulvovaginitis, and symptoms of respiratory distress with conjunctivitis. These symptoms / lesions were in agreement with descriptions of BoHV-1 outbreaks/infections in cattle (Pandey *et al.* 2000, Benoti *et al.* 2007, James and Edwards 2011).

In farm A 56%, B 38.77%, C 21.05% and in D 17.5% animals were found seropositive for BoHV-1 by ELISA. Overall 35.02% of the animals tested were seropositive for BoHV-1 antibodies (Table 1).

The overall seroprevalence recorded in breeding animals in this study (farm A, B, C and D) was 35.02%. This finding was in agreement with Jain *et al.* (2009) and Nandi *et al.* (2010) who recorded 29.21% and 35% BoHV-1 seroprevalences, respectively, in breeding animals in India. The finding of high seropositivity in this study gives a larger evidence of alarmingly widespread nature of BoHV-1 in subtropical developing countries like India, which do not practice vaccinations against BoHV-1. Further, the findings were comparable globally with the seroprevalence of 33.97% in Iran (Kargar *et al.* 2001); 37.8% in Canada (Durham and

Hassard 1990); 35.9% in Belgium (Castrucci *et al.* 1997), 40% in Netherland (Castrucci *et al.* 1997) and 38% in Poland (Ackermann and Engels 2006).

The highest seroprevalence of 56% was recorded in Farm A, which maintained breeding animals in large, open shed and were in let loose / let out, with common grazing and drinking facilities. This is attributable to the close social relationship amongst breeding animals at grazing pasture, drinking tanks and indiscriminate animal huddling during chilly weathers in open yards which facilitates direct contact between animals for easy dissemination of the virus. The seroprevalence in farm B was 38.77%, which had individual animal pens with separate feeding facility but with common watering facility. The seroprevalence was much lower in farm C at 21.05%, which followed better management practices with individual pens containing separate feeding and watering facility for each animal, thus avoiding direct contacts between animals (or their secretions in feed and water) possibly inhibiting viral entry, spread and further disseminations. The least seroprevalence of 17.75% was recorded in farm D, which had the best (on relative basis) management, hygienic and bio-security practices with spacious modern individual animal pens, separate feeding and drinking facilities for each of the animals in pen with restricted movement of personnel.

Virus isolation technique has several drawbacks in clinical diagnosis of BoHV-1 in cattle, especially the poor sensitivity (Ranganatha *et al.* 2013). In view of this, many researchers have advocated the most recent version of PCR the TaqMan probe based real time PCR as a better test in detection of BoHV-1 in clinical samples, including semen samples (Wang *et al.* 2008, Diallo *et al.* 2011, Paul and Timothy 2011) hence the present study incorporated real time PCR to detect BoHV-1 in clinical samples collected in these 4 organised farms, where most of the animals appeared healthy. The set of primers and probes designed during this study detected BoHV-1 as low as 0.001TCID₅₀/0.1ml and the assay had sensitivity of e⁻ 99% with 87.19% specificity in detection of BoHV-1. The high sensitivity in terms of detecting ultra low levels of virus of up to 0.001 TCID₅₀/0.1ml and better specificity of the real time PCR assay further demonstrates that real time PCR assay as the best test in detection of BoHV-1 in latent or carrier animals.

Subjecting the DNA extracted from clinical samples for real time PCR assay resulted in detection of BoHV-1 in 11 out of 50, 3 out of 49, and 3 out of 38 samples from Farm A, B and C respectively. None of the samples collected from Farm D were positive for BoHV-1 by real time PCR assay (Table 1). Though real time PCR does not require running the gel for PCR confirmation, as a means of authenticity a few of the real time PCR products were analyzed by agarose gel electrophoresis. The real time PCR products under gel electrophoresis yielded a specific amplicon of 71 bp (Fig. 1).

Table 1. Results of AB- ELISA and real time PCR

Farm	Sex of the animal	Total number of animals tested	Number of animals positive by	
			ELISA	Real time PCR
Farm A	Male (2) and Female (48)	50	28	11
Farm B	Male	49	19	3
Farm C	Female	38	8	3
Farm D	Male	40	7	Nil
	Total	177	62	17

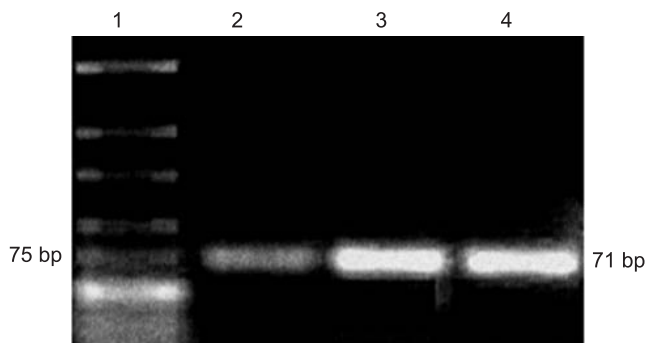


Fig. 1. Confirmation of real time PCR amplicon by gel electrophoresis. Lane M : 25bp DNA ladder; lane 1,2, 3 : Specific amplicons of 71 bp.

As evidenced in serodiagnosis, samples collected from farm A, which had close physical contacts between animals like, licking, nose to nose contacts either during common housing in large open animal sheds (since they were let loose type) or at common grazing/drinking yards (since they were let out type) probably served for better virus transmission amongst susceptible populations (Benoit *et al.* 2007), resulted in detection of virus in 11 animals. The farm D which had the best hygienic practices (on relative basis) did not yield any virus by real time PCR.

Out of 177 animal under study, 62 were seropositive to BoHV-1 antibodies, however only 17 were positive for virus by real time PCR, which is the most sensitive test prescribed by OIE (2010). This goes on to state that all seropositive animals do not excrete the virus at all the times. Due to herpes viral latency, an animal once infected develops antibodies and the animal remains infected carrier for life time. Whenever the carrier animal is subjected to stress conditions (disease/ change of weather/change of feed/place, etc.) the latent virus gets reactivated and gets excreted in the respective secretions. So an animal positive for antibody means it harbours the virus and can excrete the virus whenever they are subjected to stress conditions. Hence this study recommends the removal of seropositive animals from the herd in a breeding station.

This study further highlighted the subclinical nature of BoHV-1, since most of the breeding animals that yielded the virus by real time PCR did not show any symptoms characteristic of BoHV-1 infections in cattle. This finding was in confirmation with the works of Hage *et al.* (1996) who induced stress to a seropositive animal with dexamethasone, the animal did not produce any symptom of the disease/infection, but the swabs collected after dexamethasone treatment yielded the virus in cell cultures, emphasising the subclinical status of BoHV-1 in animals. Hage *et al.* (1996) estimated the reproduction ratio for BoHV-1 to be at least 7 in cattle herd. Nuotio *et al.* (2007) reported that 1 seropositive breeding bull transmitted the BoHV-1 infection to 9 neighbouring breeding bulls in a span of one

year in Finland. This explains the relative contagiousness of the virus in bovine population. Going by the definition of reproduction ratio and pathoepidemiology of bovine herpes virus-1, all cattle including exotic and indigenous breeds are equally susceptible for BoHV-1 infections (OIE 2010).

In view of the different modes adopted by BoHV-1 in transmission through susceptible populations, the present study finds that housing practices do have greater significance in spread and dynamics of BoHV-1 in cattle. Hence the present study through its findings and observations recommends better hygienic, bio-secured housing conditions that avoid closer animal to animal contacts, for production of pathogen free semen and other germplasms, with special reference to highly contagious BoHV-1.

ACKNOWLEDGEMENTS

Authors are thankful to all the officers and staff of breeding bull stations and bull mother farms of Government of Karnataka and Government of India for their kind co-operation during sample collections.

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