Veterinary Microbiology 171 (2014) 182–188

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short Communication

Detection of bovine herpesvirus 2 and bovine herpesvirus 4 DNA in trigeminal ganglia of naturally infected cattle by polymerase chain reaction

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ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 10 March 2014
Accepted 12 March 2014

Keywords:
Bovine herpesvirus
Ovine herpesvirus
PCR
Latency
Internal control

ABSTRACT

Establishment of latent infection within specific tissues in the host is a common biological feature of the herpesviruses. In the case of bovine herpesvirus 2 (BoHV-2), latency is established in neuronal tissues, while bovine herpesvirus 4 (BoHV-4) and ovine herpesvirus 2 (OvHV-2) latent virus targets on cells of the monocytic lineage. This study was conducted in quest of BoHV-2, BoHV-4 and OvHV-2 DNA in two hundred trigeminal ganglia (TG) specimens, derived from one hundred clinically healthy cattle, majority of them naturally infected with bovine herpesvirus 1 (BoHV-1) and bovine herpesvirus 5 (BoHV-5). Total DNA extracted from ganglia was analyzed by polymerase chain reaction (PCR) designed to amplify part of the genes coding for BoHV-2, and BoHV-4 glycoprotein B and, for OvHV-2, the gene coding for phosphoribosylformylglycinamidine synthase-like protein. BoHV-2 DNA was detected in TG samples of two (2%) and BoHV-4 DNA in nine (9%) of the animals, whereas OvHV-2 DNA could not be detected in any of the TG DNA. The two animals in which BoHV-2 DNA was identified were also co-infected with BoHV-1 and BoHV-5. Within the nine animals in which BoHV-4 DNA was detected, six were also co-infected with BoHV-1 and BoHV-5. This report provides for the first time evidence that viral DNA from BoHV-2 and BoHV-4 can be occasionally detected in TG of naturally infected cattle. Likewise, in this report we provided for the first time evidence that the co-infection of cattle with three distinct bovine herpesviruses might be a naturally occurring phenomenon.

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http://dx.doi.org/10.1016/j.vetmic.2014.03.012
0378-1135/

1. Introduction

Bovine herpesvirus type 2 is member of the subfamily *Alphaherpesvirinae*, while BoHV-4 and OvHV-2 are members of the subfamily *Gammaherpesvirinae* (Davison et al., 2009). The latent infection is a hallmark of herpesvirus' biology. In the case of alphaherpesviruses, latency is known to be established in neuronal tissues. BoHV-2 would also be expected to induce latency in nerve ganglia, although based on the usual primary target of the infection, which is the mammary gland, the virus would be expected to remain latent in inguinal nerves. Torres et al. (2009a) showed that lambs intranasally inoculated with BoHV-2 harbored latent viral DNA in trigeminal ganglia, tonsils and regional lymph nodes. So far, BoHV-4 infection has not yet been clearly associated with disease (Donofrio et al., 2007). Like other gammaherpesviruses, BoHV-4 latency is expected to be established in cells of the monocytic lineage (Osorio and Reed, 1983; Dubuisson et al., 1989), although it has also been detected in peripheral and/or central nervous system tissues (Egyed et al., 1996). OvHV-2 is the causative agent of sheep-associated malignant catarrhal fever (MCF). The virus is transmitted mainly by the respiratory route and may be shed intermittently in nasal secretions. Similarly to BoHV-4, monocytes are also presumed to be the site for latency of OvHV-2 in sheep (Li et al., 2004).

To date, serological tests for detection of BoHV-2- and OvHV-2-antibodies are not commercially available (Li et al., 2013). In addition, BoHV-2 virus isolation from infected animals is easily accomplished but essentially requires sampling of clinically apparent lesions. BoHV-4 virus isolation is common, but not routinely successful. Attempts on OvHV-2 recovery from clinical MCF cases have constantly failed. Thus, a more reliable method for identification of above mentioned viruses would have to be based on genome detection by PCR (Egyed and Bartha, 1998; Torres et al., 2009a; Li et al., 2011).

It has been previously described that BoHV-2 can establish latent infections in sensory ganglia (Letchworth and Carmichael, 1982). BoHV-4 DNA can be found in bone marrow cells at 62 d.p.i., suggesting that this virus may persist in this tissue (Egyed and Bartha, 1998). With respect to OvHV-2, this virus has been found in nasal secretions of sheep, but not in bovines (Li et al., 2004). Recently, we have investigated the presence of BoHV-1 and BoHV-5 in TG samples from cattle (Campos et al., 2009). The present study was extended in quest for BoHV-2, BoHV-4 and OvHV-2 DNA in the same TG samples in order to find out whether or not, cell types other than lymphocytes can be latently infected by BoHV-4 and OvHV-2, as well as to clearly define whether or not BoHV-2 can establish latency in TG from cattle.

2. Materials and methods

2.1. Cells and virus

The CRIB cell line, a bovine viral diarrhea virus-resistant clone derived from Madin-Darby bovine kidney cells (MDBK) (Flores and Donis, 1995), was used for virus propagation of BoHV-4. The cells were maintained in Eagle's minimal essential medium (E-MEM, Gibco) supplemented with 10% fetal bovine serum (Gibco), 10 μg/mL streptomycin (Vitalfarma), 100 μg/mL gentamicin (Gentamax®, Marcolab), and 2 μg/mL amphotericin B (Cristália). The BoHV-4 strain Movar was isolated in Europe by Bartha et al. (1966).

2.2. Collection of trigeminal ganglia

Two hundred TG specimens, derived from 100 animals of mixed breeds of both genders, with a mean age of about 4 years were collected in a slaughterhouse in the city of Pelotas, southern Rio Grande do Sul, Brazil. Details on the sampling procedure and identification of samples were provided elsewhere (Campos et al., 2009).

2.3. Extraction of total ganglion DNA

Total DNA extraction method from ganglia was described previously (Campos et al., 2009). In summary, fragments of TG of approximately 50 mg were lysed with TEN buffer [20 mM Tris–HCl (Affymetrix, USB), pH 7.4; 10 mM EDTA (Invitrogen), pH 8.0 and 200 mM $NaCl_2$ (J.T.Baker®)], 100 μg proteinase K (BioAmerica Inc.) and 1% SDS (Serva). A standard phenol (Invitrogen) extraction was performed. To check the quantity and quality of the DNA, 10 μl were loaded on agarose (Agargen) gels and compared with known quantities of lambda phage DNA (New England Biolabs). One hundred nanograms of sample DNA were added to each tube as templates for the first round PCRs that preceded nested and semi-nested reactions.

2.4. BoHV-2, BoHV-4 and OvHV-2 PCR assays

For detection of BoHV-2, BoHV-4 and OvHV-2 DNA by PCR assay, the amplification conditions of previously published PCR systems were optimized (Table 1). Total DNA extracted from ganglia was subjected to PCR assay designed to amplify part of the genes coding for BoHV-2 and BoHV-4 glycoprotein B (gB) and, for OvHV-2, the gene coding for phosphoribosylformylglycinamidine synthase-like protein. The assays were carried out in two steps: in a first round of reactions, 100 ng of total DNA and fixed amounts of an internal control (IC; see below for details) were added; then, in a second round of reactions, 1 μl of the first reaction was used as template. All reactions of the first PCR assay, including apparently negative results, were subjected to nested polymerase chain reaction (nPCR) (for BoHV-4 detection) and to semi-nested polymerase chain reaction (snPCR) (for BoHV-2 and OvHV-2 detection). Table 1 shows the targeted genes, primers and sizes of the expected products. All amplification assays were performed in a Mastercycler apparatus (Eppendorf), in a final volume of 25 μl. Each reaction tube contained 1 mM $MgCl_2$ (Invitrogen), 0.2 μM of each primer (IDT), 10% dimethylsulfoxide (DMSO; Nuclear), 1 U Taq DNA polymerase (Invitrogen), 10% PCR buffer (Invitrogen) and 0.4 mM deoxynucleoside triphosphates (GE Healthcare). Amplification reactions were performed under the following