Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org http://ejvs.selcuk.edu.tr

SHORT COMMUNICATION

Molecular investigation of Bovine Herpesvirus Type 1 from aborted small ruminant foetuses

Murat Sevik[1], Oguzhan Avci[2]*

[1]Molecular Microbiology Laboratory, Veterinary Control Institute,
[2]Department of Virology, Faculty of Veterinary Medicine, University of Selcuk, Konya, Turkey
Received: 10.09.2014, Accepted: 18.10.2014
*oavci@selcuk.edu.tr

Öz

Şevik M, Avci O. Atık küçük ruminant fötuslarında Bovine Herpes Virus-1'in moleküler olarak araştırılması.

Abstract

Sevik M, Avci O. Molecular detection of Bovine Herpesvirus Type 1 from aborted small ruminant foetuses.

Eurasian J Vet Sci, 2015, 31, 1, 57-59
DOI: 10.15312/EurasianJVetSci.201518478

Bu çalışmada atık küçük ruminant fötus dokularında, Bovine Herpes Virus-1 (BoHV-1) varlığının PCR tekniği kullanılarak belirlenmesi amaçlandı. Bu amaçla İç Anadolu Bölgesi'ndeki farklı illerden (Konya, Aksaray ve Niğde), BoHV-1 seropozitif sığırlar ile bir arada yaşayan, her biri farklı sürülerdeki küçük ruminantlara ait atıklardan (43 koyun ve 23 keçi) iç organ (akciğer, karaciğer ve dalak, toplam n=198) örnekleri toplandı. Glikoprotein D (gD) genine spesifik polimeraz zincir reaksiyonu (PZR) kullanılarak, BoHV-1 varlığı araştırıldı. Doku örneklerinin hiçbirinde BoHV-1 varlığı tespit edilemedi. Elde edilen sonuçlara dayanarak, İç Anadolu Bölgesi'nde BoHV-1'in küçük ruminant abort vakalarında rol oynamadığı ifade edilebilir.

Anahtar kelimeler: Bovine Herpesvirus 1, küçük ruminant, abort, PCR

The aim of this study was carried out to determine the presence of Bovine Herpes Virus-1 (BoHV-1) in aborted small ruminant foetus' tissues by using Polymerase Chain Reaction (PCR) technique. For this purpose, aborted tissue samples (liver, lung, and spleen, totally n=198) were collected from small ruminants (43 sheep and 23 goats), each from different flocks and lived together with BoHV-1 seropositive cattle, in Central Anatolia region (Konya, Aksaray, and Niğde provinces) of Turkey. Glycoprotein D (gD) gene based PCR was used for the detection of BoHV-1. All tissue samples were detected as negative for BoHV-1 DNA. According to results, it can be expressed that BoHV-1 is not play a role in abortion cases in small ruminants in Central Anatolia Region.

Keywords: Bovine Herpesvirus 1, small ruminant, abortion, PCR

Bovine herpesvirus-1 (BoHV-1), classified as a member of the herpesvirus family, is the causative agent of infectious bovine rhinotracheitis (IBR), a disease of the upper respiratory tract in cattle (Mweene et al 2003). It can also cause abortion in cattle (Muylkens et al 2007). Latent infection may occur in closed herds and latent virus can be reactivated by different stressful conditions such as infections, corticosteroid applications or transportation (Muylkens et al 2007).

BoHV-1 leads to significant economic losses on the farming industry due to abortion and reproductive problems (Mweene et al 2003). It has been reported that BoHV-1 primarily infects cattle, but also can infects sheep and goats (Goyal et al 1988). Furthermore, cross-species infections between bovine and caprine herpes viruses have been demonstrated in previous studies (Lehmkuhl et al 1985, Wafula et al 1985, Yesilbag et al 2003).

Various laboratory methods such as virus isolation in cell culture (Mahmoud and Ahmed 2009), immunoperoxidase (Smith 1997), immunofluorescent antibody (OIE 2008), enzyme-linked immunosorbent assay (ELISA) (Parreno et al 2010, Raaperi et al 2010) have been used for the serological and virological diagnosis of BoHV-1. As an alternative to these methods, several PCR techniques were developed for the rapid and specific detection of BoHV-1 (Mylissa et al 2008, Mahmoud and Ahmed 2009).

The isolation of BoHV-1 from Peninsular bighorn sheep (Ovis Canadensis Cremnobates) (Clark et al 1993) and the detection anti BoHV-1 antibodies in sheep in Mali (Maiga and Sarr 1992) have been demonstrated. Therefore, these data suggest that BoHV-1 may play role in abortion in small ruminants. So, in this study we investigated the role of BoHV-1 on abortion cases in small ruminant that farmed with BoHV-1 seropositive cattle.

In the first stage, a list of all available farms that have IBR in the background and cattle farmed with small ruminants was obtained from district animal health offices in 3 different provinces (Konya, Aksaray, and Niğde) in Central Anatolia region of Turkey, and then the required number of serum samples was selected by using simple random sampling. Serum samples (n=188) were tested for the detection of glycoprotein E (gE) specific antibodies to BoHV-1 by using commercial ELISA test kit (IDEXX IBR gE Ab test, Institut Pourquier, Montpellier, France) according to manufacturers' instruction. Tissue samples (lung, liver, and spleen) were collected from cases of abortion in small ruminants (n=66; 43 sheep and 23 goats) from determined as BoHV-1 seropositive farms (n=91) between January 2011 to January 2012.

Viral DNA was extracted from tissue samples (25-30 mg) using a robotic extraction method (MagNA Pure LC 2.0 System, Roche Applied Science, Indianapolis, IN, USA) with the Magna Pure LC total nucleic acid isolation kit. PCR was performed in a total volume of 50 µL containing 25 µM of each primer, 0.25 mM of deoxyribonucleotide triphosphate (dNTPs), 2 mM $MgCl_2$, 0.25 µL of Taq DNA polymerase and 5 µL volume of sample DNA; sterile ribonuclease-free double distilled water was added to give a total volume of 50 µL. Primers which amplify approximately 343 bp fragment from the gD gene described by Smits et al. (2000) were used. The following cycling program was performed in a thermocycler (MJ Research Inc., USA): 35 cycles of 15 s at 94°C, 15 s at 67°C and 60 s at 72°C. A final extension time of 5 minutes at 72°C was included at the end of last cycle. The PCR products were analyzed in 1.5% agarose gel after electrophoresis at 90 V for 45 minutes and visualized by with ethidium bromide staining. PCR was found negative in all of lung, liver and spleen samples tested in each of tissue samples (n=198).

Diseases of the respiratory tract are common in all species and different viral and bacterial agents have now been recovered from the respiratory tracts of animals (Giangaspero et al 2013). BoHV-1 is an important viral agent that causes upper respiratory disease in cattle. Other ruminant species are also thought to be susceptible to BoHV-1 infection (OIE 2008). In previous studies, antibodies against BoHV-1 in sheep was reported from different countries such as Nigeria (Taylor et al 1977), Canada (Elazhary et al 1984), USA (Goyal et al 1988), India (Singh et al 2001), Egypt (Mahmoud and Ahmed 2009) and Turkey (Yesilbag and Dagalp 2006, Albayrak et al 2007, Ataseven et al 2010). Also, BoHV-1 was isolated from a lamb with respiratory symptoms (Trueblood et al 1978). However, only cattle determined as reservoirs of BoHV-1 (OIE 2008).

The infection is usually confirmed by serological assays such as ELISA designed to detect the presence or absence of antiviral antibodies. Neutralization and isolation of virus in cell culture is technically difficult and time-consuming and thus is not suitable as a routine diagnostic assay. Therefore we used PCR for detection of BoHV-1. PCR provides rapid, sensitive and reliable diagnosis of the disease (OIE 2008). Spesific antibodies against BoHV-1 in goats in Turkey were reported by some researchers (Yesilbag et al 2003, Ataseven et al 2010). However, there is no information that BoHV-1 can cause abortion in small ruminants in Turkey.

Findings from our study suggest that BoHV-1 does not play a role in abortion cases in small ruminants. Though, BoHV-1 infection risk in small ruminants should be taken into consideration.

Acknowledgement

This study has been presented in XI. Veterinary Microbiology Congress as a poster presentation, 21-24 October 2014, Antalya, Turkey.

References

Ataseven VS, Başaran Z, Yilmaz V, et al., 2010. Seroprevalence of Parainfluenza Virus-3 (PIV-3) and Bovine Herpesvirus Type 1 (BHV-1) infections in goats of Van region. YYU Vet Fak Derg, 21, 7-9.

Albayrak H, Yazici Z, Gumusova SO, 2007. Seroprevalence to bovine herpesvirus type 1 in sheep in Turkey. Vet Arhiv, 77, 257-263.

Clark RK, Whetstone CA, Castro AE, et al., 1993. Restriction endonuclease analysis of herpesviruses isolated from two peninsular bighorn sheep *(Ovis Canadensis Cremnobates).* J Wildlife Dis, 29, 50-56.

Elazhary MASY, Silim A, Dea S, 1984. Prevalence of antibodies to bovine respiratory syncitial virus, bovine viral diarrhoea virus, bovine herpesvirus-1, and bovine parainfluenza-3 virus in sheep and goats in Quebec. Am J Vet Res, 45, 1660-1662.

Giangaspero M, Savini G, Orusa R, et al., 2013. Prevalence of antibodies against Parainfluenza virus type 3, Respiratory syncytial virus and bovine Herpesvirus type 1 in sheep from Northern Prefectures of Japan. Vet Ital, 49, 285-289.

Goyal SM, Khan MA, McPherson SW, et al., 1988. Prevalance of antibodies to seven viruses in a flock of ewes in Minnesota. Am J Vet Res, 49, 464-467.

Lehmkuhl HD, Cutlip RC, Bolin SR, et al., 1985. Seroepidemiologic survey for antibodies to selected viruses in the respiratory tract of lambs. Am J Vet Res, 46, 2601-2604.

Mahmoud MA, Ahmed SA, 2009. Prevalence of Bovine Herpesvirus-1 in sheep and goats in Egypt. Global Veterinaria, 3, 472-479.

Maiga S, Sarr J, 1992. Epidemiological survey of the main respiratory viruses of small ruminants in Mali. Rev d'Elevage Med Vet Pays Trop, 45, 15-17.

Muylkens B, Thiry J, Kirten P, et al., 2007. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Vet Res, 38, 181-209.

Mweene AS, Fukushi H, Pandey GS, et al., 2003. The prevalance of bovine herpesvirus in traditional cattle in Southern Province, Zambia. Rev Sci Tech, 22, 873-877.

Mylissa SD, Marley MD, Givens PK, Galik KP, Riddell DAS, 2008. Development of a duplex quantitative polymerase chain reaction assay for detection of bovine herpesvirus 1 and bovine viral diarrhea virus in bovine follicular fluid. Theriogenology, 70, 153-160.

OIE 2008. Principles of validation of diagnosis assays for infectious diseases. In: Annual of diagnostic tests and vaccines for terrestrial animals, Paris, France. Accessed at: 05.09.2014

Parreno V, Romera SA, Makek L, et al., 2010. Validation of an indirect ELISA to detect antibodies against BoHV-1 in bovine and guinea-pig serum samples using ISO/IEC 17025 standards. J Virol Methods, 169, 143-153.

Raaperi K, Nurmoja I, Orro T, et al., 2010. Seroepidemiology of bovine herpesvirus 1 (BHV1) infection among Estonian dairy herds and risk factors for the spread within herds. Prev Vet Med, 96, 74-81.

Singh RP, Bhat MN, Sastry KNN, 2001. Studies on the prevalance of antibodies to BHV-1 in sheep. Indian Vet J, 78, 467-470.

Smits CB, Van Maanen C, Glas RD, De Gee AL, Dijkstrab T, Van Oirschot JT, Rijsewijk FA, 2000. Comparison of three polymerase chain reaction methods for routine detection of bovine herpesvirus 1 DNA in fresh bull semen. J Virol Methods, 85, 65-73.

Smith KC, 1997. Herpesviral abortion in domestic animals. Vet J, 153, 253-268.

Taylor WP, Okeke ANC, Shidali NN, 1977. Prevalence of bovine viral diarrhea and infectious bovine rhinotracheitis antibodies in Nigerian sheep and goats. Trop Anim Health Prod, 9, 171-175.

Trueblood MS, Swift BL, McHolland-Raymond A, 1978. A bovine herpesvirus isolated from sheep. Can J Comp Med, 42, 97-99.

Wafula JS, Mushi EZ, Wamwayi H, 1985. Reaction of goats to infection with infectious bovine rhinotracheitis virus. Res Vet Sci, 39, 84-86.

Yesilbag K, Bilge-Dagalp S, Okur-Gümüşova S, et al., 2003. Studies on herpesvirus infections of goats in Turkey: Prevalence of antibodies to bovine herpesvirus 1. Rev Med Vet, 154, 772-774.

Yesilbag K, Dagalp SB, 2006. Seroprevalance of bovine herpesvirus 1 infection in sheep in Turkey. AÜ Vet Fak Derg, 53, 141-143.