



Detection of antibody against infectious bovine rhinotracheitis glycoprotein gE in aborted cattle in Mashhad, Iran

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Received xx Oct 2009; accepted xx Apr 2009

ABSTRACT

Infectious Bovine Rhinotracheitis is a highly contagious disease caused by the bovine herpes virus-1 (BoHV-1), resulting in significant losses to livestock around the world. BoHV-1 is a major pathogen of cattle, primarily associated with respiratory/genital tract infections and abortion. In the present study, we determined the presence of antibodies in 120 serum samples of cattle with the history of abortion in different period of pregnancy from different industrial dairy herds in Mashhad. Also we tested 30 samples from normal cattle with no history of abortion as negative control. The presence of antibodies against infectious bovine rhinotracheitis was investigated by enzyme-linked immunosorbent assay (ELISA). The results showed that seroprevalence of IBR in aborted cattle were 70% (84 samples). From these positive samples, 11 (13.09%), 42 (50.00%) and 31 (36.91%) samples were associated to the first, second and third trimester of pregnancy, respectively. From these seropositive cattle (84 samples), 12 (14.28%) samples were associated with stillbirth and 7 (8.33%) samples were related to mummified fetus. From 84 positive samples, 59 (70.24%) were related to cattle between 2-5 years old and 25 (29.76%) were associated to cattle more than 5 years old. In negative control group, 5 samples showed antibody against IBR antigen.

Keywords: Abortion, Antibody, Cattle, ELISA, Infectious bovine rhinotracheitis

INTRODUCTION

Infectious Bovine Rhinotracheitis is a highly contagious disease caused by the bovine herpes virus-1 (BoHV-1), resulting in significant losses to livestock around the world (Nardelli *et al* 2008). The disease is noticeable in many, but not all countries. Bovine herpesvirus 1 (BoHV-1), classified as an alphaherpesvirus, is a major

pathogen of cattle. Only a single serotype of BoHV-1 is recognized, but subtypes of BoHV-1 have been described on the basis of restriction enzyme cleavage patterns of viral DNA. Animals with a latent BoHV-1 infection may serve as a source of infection for susceptible animals if and when the virus is reactivated (Engels & Ackermann 1996). Primary infection is accompanied by various clinical manifestations such as infectious bovine rhinotracheitis, abortion, infectious pustular vulvovaginitis, and systemic infection in neonates. Abortion is a consequence of a respiratory BoHV-1

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infection of a seronegative cow (Ackermann & Engels 2006). Naturally occurring BoHV-1 abortions are usually observed at 4 to 8 months of gestation although experimental virus inoculation of heifers prior to 3 months induces embryonic death (Muylkens *et al* 2007). Like other alphaherpesviruses, an essential intrinsic characteristic of BoHV-1 is that the virus remains in a latent state in ganglionic sensory neurons following infection and can be reactivated by different stress stimuli. There are many serological tests for detection BoHV-1 antibodies. The virus neutralization test (VNT), gB-ELISAs and indirect enzyme-linked immunosorbent assays (ELISAs), cannot differentiate between infected and vaccinated animals (Kramps *et al* 2004). Serum neutralization tests and various ELISA are routinely used for BoHV-1 antibody detection (Van Oirschot 2000). The aim of this study is detecting antibodies against glycoprotein gE of bovine herpes virus-1 (BoHV-1) in cattle with abortion by ELISA.

MATERIAL AND METHODS

Sample Collection. One hundred and twenty blood samples were collected from the cows with the history of abortion in different trimester of pregnancy from different industrial dairy herds in Mashhad, Iran. The blood samples were centrifuged at $2000 \times g$ at room temperature for five minutes to separate sera. Serum samples were stored at -20°C until used.

Indirect ELISA assay. Serum samples were tested for the presence of anti-gB antibodies using Antibody Test kit manufactured by IDEXX (HerdChek, IDEXX Laboratories, Westbrook, ME, USA), in a 96-well micro titration plates. Tests were carried out in duplicate. According to the manual, serum samples were diluted (1:1) by wash solution and 100 μl of diluted sera was loaded into wells and incubated for 2 hours at 37°C . Positive and negative control sera were used as indicated in the kit. The

wells were washed five times with 300 μl of wash solution. Following the final washing, the plates slapped vigorously, well down on a bench top which covered with paper towels. Then, 100 μl of anti IBR-gB Horseradish Peroxidase (HRP) conjugated was loaded into all the wells and incubated for one hour at room temperature. The plates were washed as described above to remove the excess conjugate. For color development, 100 μl of 3,3', 5,5'-Tetramethyl benzidine (TMB) substrate solution (TMB/H₂O₂ solution) was added to each well and incubated for 10 minutes at room temperature at darkness. The reaction was terminated by the addition of 100 μl of stop solution to each well. The absorbance at 450 nm was monitored in ELISA reader.

Calculation. Calculations for test samples were analyzed as follow for BoHV1 antibody: The presence or absence of antibody to IBR-gB in the sample is determined by the blocking percentage for each sample.

$$\frac{\text{NCx}^- \text{ A450} - (\text{OD Sample}) \text{ A450} \times 100(\%)}{\text{NCx}^- \text{ A450}}$$

NCx⁻ represent negative control mean. OD represents absorbance of each sample in 450 nm. According to manual, samples with blocking less than 45% were classified as negative, samples with blocking equal or greater than to 45% but less than 55% were classified as suspected and must be retested, and samples with blocking equal or greater than 55% were considered as positive for IBR antibodies.

Statistical analysis. Proportion of seropositivity was compared between aborted and healthy cattle using Chi-square test. Also, in aborted cattle, association among age, time of abortion and type of abortion with proportion of seropositivity analyzed using the above test.

RESULTS

The presence of antibody against infectious bovine rhinotracheitis in sera from 120 cattle with

history of abortion was investigated by indirect ELISA. Unfortunately, undiluted samples could not be used in the indirect ELISA due to unacceptably high background reactions. From 120 serum samples, 84 (70%) samples were seropositive to BoHV-1. In negative control, 5 (16.66%) samples showed antibody against IBR antigen. The level of antibody with blocking less than 45% was considered as negative. Proportion of seropositivity in aborted cattle was significantly higher than healthy cattle ($P < 0.001$). From positive samples in aborted cattle, 11 (13.09%), 42 (50.00%) and 31 (36.91%) samples were associated to the first, second and third trimester of pregnancy, respectively. From 84 seropositive samples in aborted cattle, 12 (14.28%) samples were related to stillbirth. From these, 2 (16.67%), 7 (58.33%) and 3 (25%) samples were related to first, second and third trimester of pregnancy. Also in seropositive samples from aborted cattle, 7 (8.33%) samples associated with mummified fetus. From these samples, 1 (14.29%), 3 (42.86%) and 3 (42.86%) were associated to the first, second and third trimester of pregnancy, respectively. From 84 positive samples in aborted cattle, 59 (70.24%) were related to cattle between 2-5 years old and 25 (29.76%) were associated to cattle more than 5 years old. In negative group, 1 (20%) sample was related to cattle between 2-5 years old and 4 (80%) were associated to cattle more than 5 years old. In aborted cattle, association among age, time of abortion and type of abortion with proportion of seropositivity showed in (Table 1).

DISCUSSION

In this study, the presence of antibody against BoHV-1 in serum samples of cattle was tested by Indirect ELISA. Our results showed that the seroprevalence of BoHV-1 was high (70%) in cattle in Mashhad and the remarkable difference were found among herds. The methods used in

BoHV-1 antibody test might affect the accuracy of the results, although using gB-antibody ELISA for detecting is more sensitive than the others tests (Kramps *et al* 2004). In different parts of Iran, 9968 serum samples of cattle for the prevalence of antibody against bovine herpes virus-1 were examined by virus neutralization test (Kargar Moakhar *et al* 2001). From these samples, 33.97% was positive, which indicated that IBR infection is widely distributed among the bovine population. The prevalence of antibody to the bovine herpes virus (IBR) was determined in Uremia by examining 121 serum samples of buffalo by serum neutralization test and antibody against IBR was found in 5 samples (Kargar Moakhar *et al* 2002). Hematzadeh *et al* (2002) reported that, 874 serum samples were taken from different township of Chaharmahal-Bakhtiary province in Iran.

Table 1. Percentage of seropositivity based on age, time and type of abortion in aborted cattle

Variable	Level	Number of serum positive	% of Serum positive*	P value
Age	2-5 years	59	72 ^a	0.493
	5> years	25	66 ^a	
Time of abortion	First trimester of pregnancy	11	78.5 ^a	0.758
	Second trimester of pregnancy	42	68.8 ^a	
	Third trimester of pregnancy	31	68.8 ^a	
	Stillbirth	12	100 ^a	
Type of abortion	fetus			0.009
	Mummified fetus	7	100 ^{ab}	
	abortion	65	64.3 ^b	

* Percentage with different superscript letters is significantly different ($P < 0.05$).

All of those samples were tested in serum neutralization test. Infection rate reported as 47.68%. The infection rates in cows with regular vaccination and cows without vaccination programmers were 46.6%, and 48.5% respectively. The infection rates in cows with abortion were

65.5% and with no abortion were 46.7%. In another study by Raoofe *et al.* (2004), serum samples were collected from 402 sheep during four seasons in years 2002-2003 in all townships of Chaharmahal-Bakhtiary province by cluster random sampling method. Sera were tested for antibodies against IBR virus and seropositive samples were 10.7% (43 samples). According to Wang *et al* (2005) study, there is no outbreak of BoHV-1 infection in China, however, seroprevalence rates in some herds and the levels of seroprevalence in Chinese cows ranged from 5.4% to 68.7% (Xiao *et al* 2004). The overall seroprevalence in china was 35.8%, even higher than that (21.7%) of samples from the imported cows. However, the application of these data is restricted because of the biased sampling, limited sampling area and size (Yan *et al* 2008). A total of 2754 bovine blood samples were examined for BoHV1 antibodies in three different BoHV-1 ELISA tests. Although the specificity of the gB-ELISAs was high in this comparative study, it should be noted that with this type of test probably false-positive results will occasionally be obtained when applied routinely (Mars *et al.*, 2000; Isa *et al.*, 2003). Such false-positive reactions could not be well explained, although a certain effect of testing “fresh” sera might have played a role, as was suggested for the BoHV1 gE-ELISAs (Beer *et al.*, 2003). There is undoubtedly a link between incidence of seroconversion and herd size/presence of positive animals, but it is difficult to assess the herd size and number of positive animals, because these two parameters covariate with one another. In any case, positive farms were at higher risk than negative farms of similar size: the absolute number of positive animals (growing as herd size becomes larger) surely contributes in increasing the risk (Nardelli *et al* 2008). Our results showed that high level of antibody to IBR in aborted cattle, which was determined by indirect ELISA with detecting antibodies against glycoprotein of BoHV-1

infection. Based on these results, we can not establish the relationship between abortion and IBR. So interpretation is difficult as the abortion is commonly found so long after the IBR infections occurred. However, configuration of disease by serological means must be demonstrated by either seroconversion or significant rise in antibody levels. It has been reported that if there are >15 – 20% seropositive animals in the population, vaccination is the most realistic strategy to eradicate IBR/IPV (Nardelli *et al.*, 2008). Therefore, there is a need for Iranian government to take strict precautions against potential BoHV-1 outbreak. This study provided baseline data for future studies as it was indicated that aborted cattle may be exposed to IBR.

Acknowledgment

This study was supported by a grant from Center of Excellence in Ruminant Abortion and Neonatal Mortality, Faculty of Veterinary Medicine of Ferdowsi University of Mashhad.

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