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Short Communication

Serological survey of pestivirus infection of small ruminants in Ahvaz, Iran

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ABSTRACT

In order to study the seroprevalence of pestivirus infection in small ruminants of Ahvaz at Khouzestan province of Iran, 148 sheep sera and 143 goat sera were randomly collected and tested by seroneutralization. Seroneutralization was performed by NADL strain of Bovine viral diarrhea virus genotype 1. The results indicated the overall seroprevanelces of 46.62% in sheep and 32.871% in goats. Prevalence of pestivirus antibody showed an increase with respect to age of the animals but a significant difference was only observed between sheep <2 years and sheep >4 years. This study is the first report of pestivirus infection of goat in Iran.

Keywords: Pestivirus, Antibody, Sheep, Goat

INTRODUCTION

Border disease (BD) is a congenital virus disease of sheep, characterized by barren ewes, abortion, stillbirths and the birth of small weak lambs, some of which show tremor, abnormal body conformation and hairy fleeces. Border disease also occurs in goats. There appears, however, to have been only one single report of the natural disease in this animal (Nettleton 2004).

Border disease virus (BDV) is classified in the genus pestivirus within the family flaviviridae. The family comprises three other genotypes, namely: Bovine viral diarrhea virus 1 (BVD-1), BVD-2 and Classical swine fever virus (CSFV). There is extensive antigenic cross reactivity among these genotypes and with the exception of CSFV. They can cross the host species barrier. While CSFV is predominantly restricted to pigs, BDV affects sheep, goats and pigs and BVD genotypes 1 and 2 can infect cattle, sheep, goats and pigs (Paton *et al* 1995, Hurtado *et al* 2003). In fact, pestiviruses of cattle and pigs can also cause BD in sheep and the most serious

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threat comes from cattle (Carlsson 1991), since they are the principal hosts of pestiviruses. So far, there have been several reports of the prevalence of pestivirus antibody in cattle of Iran (Sedighi nedjad 1996, Hemmatzadeh *et al* 2001, Haji Hajikolaei & Seyfiabad Shapouri 2007), but information we have about pestivirus infections in small ruminants of the country is limited to a single study, carried out on sheep from some provinces (Keyvanfar *et al* 2000). The present work was performed to study the prevalence of pestivirus antibody in sheep and goat population in Ahvaz at Khouzestan province of Iran.

MATERIALS AND METHODS

Serum samples. One hundred forty-eight blood samples from sheep and 143 samples from goats were randomly collected from five different locations around Ahvaz. After coagulation, sera were separated by centrifugation and stored at -20 ºC until serological testing.

Virus and cell culture. The NADL strain of BVD-1 was used as a reference pestivirus in a serum neutralization test. The virus was propagated in Bovine turbinate (BT) cells, cultured in Doulbecco's Modified Eagle Medium (DMEM) supplemented with 5% horse serum. Virus stock was stored in 0.5 ml aliquots at -70 ºC and tittered before using for neutralization test.

Serum neutralization test. Serum neutralization test using the NADL strain of BVD-1 was performed in BT cells. In brief, sera were heat inactivated at 56 ºC and diluted 1/4 in DMEM containing 5% horse serum. After diluting, 25 µl of each serum was mixed with 25 µl (100 $TCID_{50}$) of the virus (to obtain a final dilution of 1/8) and incubated for 1 hour at 37 ºC. Thereafter, serum-virus mixtures were transferred to 96 wells cell culture plates and $5x10^4$ BT cells/well, was added. Each serum was tested in duplicate. Plates were incubated at 37 ºC for 5 days and observed daily for the presence of cytopathic effects, compared to cell and virus controls.

Statistical analysis. The Chi-Square and Fisher's exact tests were used to test the significant differences of results.

RESULTS AND DISCUSSION

The results of the study are indicated in tables 1 and 2. A total of 69 (46.62%) of 148 sheep sera were positive for pestivirus antibody (table 1). Overall seroprevalence showed an increase with respect to age and a significant difference ($P<0.05$) was observed between sheep <2 years and sheep >4 years (table 1).

Table 1. Prevalence of seropositive sheep to Pestivirus, according to age groups.

Age groups	Seropositive sheep		Seronegative sheep		tested
	number	%	number	%	
<2 years	5	25	15	75	20
2 years	11	44	14	56	25
3 years	9	45	11	55	20
≥ 4 years	44	53	39	47	83
Total	69	46.62	79	53.38	148

Table 2. Prevalence of seropositive goats to Pestivirus, according to age groups.

Age groups	Seropositive goats		Seronegative goats		tested
	number	%	number	%	
<2 years	8	25.8	23	74.2	31
2 years	10	32.25	21	67.75	31
3 years	12	36.4	21	63.6	33
≥ 4 years	17	35.4	31	64.6	48
Total	47	32.87	96	67.13	143

Among 143 goat sera, 47 (32.87%) were found to be positive in serum neutralization test (table 2). The highest seroprevalence was observed among 3 yesrs old animals but, unlike the results observed for sheep sera, seroprevalence was not significantly different according the age groups of goats. Compared to goat's sera, sera of sheep older than 2 years showed a higher percent of seropositive animals, but the

differences were not significant. The present study shows the prevalence of pestivirus infection in the sheep and goats of Ahvaz in kouzeastan province of Iran. Pestivirus infection of sheep in some provinces of Iran, including kouzestan, has been previously reported by Keyvanfar *et al* (2000), but so far, there has not been any investigation on pestivirus infection of goat in Iran. Therefore, the present work is the first report of pestivirus infection of goat in Iran. Serological survey data from several countries have shown the presence of pestivirus infections in small ruminants. Reports from many countries indicate that the prevalence of pestivirus antibody in sheep is varying from about 5 to 50 percent (Nettleton 2004). More limited studies performed on goat sera have revealed that about 2 to 25 percent of goats have antibody to pestivirus (Loken 1995, Krametter-Froetscher *et al* 2006). The overall seroprevanelces of pestivirus antibody (46.62% in sheep and 32.87% in goats) that we found in the small ruminants of the region are among the highest levels reported so far. Studies of BDV infection in sheep indicate that transmission is dependent on the degree of contact between infected and non-infected animals (Barlow *et al* 1980, Nettleton *et al* 1992). Therefore, geographical variation of seroprevalence between different countries, and even between different flocks in a country, may be related to flock density and farm management practices, as discussed previously (Lamontagne & Roy 1984, Loken *et al* 1991, Graham *et al* 2001).

The results of our study indicated an increase of seroprevalence by increasing the age of the sampled animals and the difference was significant ($P<0.05$) between sheep <2 years and sheep >4 years. This could be explained by a higher chance of older animal for exposure to virus. In this study, we did not analyzed the results based on the sampled flocks, but it has been demonstrated that age-specific antibody patterns could differ according to the presence or absence of persistently infected (PI) animals in flocks (Berriatua *et al* 2004). Seroprevalence in flocks with PI sheep has been high in all age groups but in flocks with past evidence of PI sheep, seroprevalence has been high in old animals and much lower in young sheep. Establishment of PI kids in goats seems be more difficult than in sheep or cattle (Depner *et al* 1991). Therefore, sources of infection for our mixed flocks of sheep and goats, are probably persistently infected (PI) sheep or in contact cattle. However, some seroconversions could also be the result of infection from non-PI animals, because it has been demonstrated that BVDV can circulate in herd cattle for 2-2.5 years in the absence of PI animals (Barber and Nettleton 1993, Moerman *et al* 1993). Clinical BD can significantly reduce productivity of animals and cause economic losses. Despite a seroprevalence of pestivius infections we showed, so far clinical manifestations of BD in lambs have not been reported from this region. This situation may be explained by the facts that clinical diagnosis of BD is difficult, only a small number of lambs show typical clinical signs and pestivirus strains are variable in the virulence (Lamontagne & Roy 1984). However, abortion storms with unidentified etiology(s) which are frequently observed in sheep flocks of the region may be related to pestiviruses, because infection with BDV has also been considered as a major cause of abortion in sheep (Berriatua *et al* 2004).

In conclusion, the result of this study suggests that pestivirus infection must be taken into consideration as an etiological agent for abortions or birth of weak lambs or goat in Khouzeastan province of Iran. Moreover, further studies are necessary to isolate and characterize the regional pestiviruses.

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