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Seroepidemiology of Border Disease and Risk Factors in Small Ruminants of Shiraz Suburb, Fars Province, South of Iran

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Abstract: Border disease virus (BDV) is a member of the genus *Pestivirus* in the family *Flaviviridae* which causes abortion, wool deformation, congenital malformations and weak lamb syndrome in small ruminants and produces enormous economic losses. The present study was conducted to determine the seroprevalence of the disease and some risk factors that might be related to it. A total of 200 serum samples were collected from 13 flocks including nomadic animals (70%) and resident flocks (30%) using a cluster random sampling method with equal proportion of sheep and goats during three last months of 2010. Some data such as age, species, exposure to other flocks, density and female replacement origin were reported in a question sheet. Totally, 17% of the samples were positive (95% CI=11-23). The results showed that BDV probably is related to ovine abortion in the studied region (P=0.09). Also, ewe and doe replacement from other flocks might be related on seroprevalence of the disease (P=0.08). By the way, the lower density of animals can probably make the lower seropositive animals in the flock (P=0.09).

Key words: Border disease · Seroepidemiology · Small Ruminant · Shiraz · Iran

INTRODUCTION

Border disease (BD) is a world- wide occurring viral disease of sheep and goats which causes economically important losses [1]. Border disease is an endemic disease of small ruminants and was first reported during 1959 from the border region of England and Wales [2]. It has subsequently been reported from most of the major sheep and goats producing countries and probably occurs in all [1]. BD can be caused by three species of the four currently recognized members of the genus Pestivirus, family Flaviviridae: Border disease virus (BDV), Bovine viral diarrhea virus species 1 (BVDV-1) and BVDV-2 [3, 4]. Based on the most recent knowledge ovine pestiviruses are segregated at least into six clusters [4-6]. Additionally, BDV strains in the two different phylogenetic groups determined in Tunisia [7] and Italy [8]. Clinical signs in sheep include barren ewes, abortions, malformations, stillbirths, birth of small weak lambs and persistent infections of the offspring. Affected lambs can show tremor, abnormal body conformation and hairy fleeces (so-called 'hairy-shaker-' or 'fuzzy' lambs syndrome) [9-11]. BDV have also caused mucosal disease-like lesions in sheep [12]. In addition, BDV infections in pregnant goats result nearly exclusively in abortions and malformations in fetuses and neonates. Therefore, in contrast to sheep, persistently infected goats have not been reported [10]. Besides the important economical losses caused as primary pathogens, pestiviruses may compromise the normal immune response to other pathogens and increase the severity of other infections in sheep [13]. Serological studies have shown seroprevalences ranging between 5 and 50% for various countries and regions within countries [11].

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Within Iran, Fars province is a major sheep and goat producing province. Considering this fact that there is a lack of information on the seroprevalence of border disease in suburb of Shiraz, the study was carried out to clarify different aspects of the disease in sheep and goat population of the region.

MATERIALS AND METHODS

The cross-sectional study was conducted on sheep and goat herds in Shiraz suburb of Fars province, southern Iran. Two small ruminant production systems are described in this area. One is nomadic system; herds migrated in spring and fall following find better feedstuffs and weather, another system is resident; owners fed herds with local grazing and complementary feedstuffs for cold season. In this study, 200 serum samples were collected from 13 flocks including nomadic animals (70%) and resident flocks (30%) using a cluster random sampling method with equal proportion of sheep and goats during last three months of 2010. The population of the tested sheep and goats herd complexes was between 80 and 350. The total small ruminant population under the study was 2490. All of the farmers used natural breeding in their herds. Sheep and goats having abortus until one month were used in the study. Of these samples, 138 and 62 samples belonged to nomadic and resident flocks, respectively. A questionnaire asking for the epidemiological data of the flock and the animal was discussed with the farmers and their practitioners, in a personal interview. A summary of flock characteristics and management practices potentially associated with BDV infections are presented in Table 1.

Blood samples were taken from the jugular vein into a plain vacutainer tube. The samples were allowed to clot at room temperature for 40 minutes and then centrifuged at 3000 g for 10 minutes and serum was collected and stored at -20°C until testing. Undiluted serum samples were tested for antibodies to BDV using a commercially available indirect ELISA (SVANOVIR BD-Ab ELISA; Svanova Biotech) according to the manufacturer's instructions. The ELISA was designed for detection p125/p80 that is a highly conserved non-structural pestivirus protein and it is considered to be the protein of choice for developing a diagnostic pestivirus antibody ELISA [14]. Relative to the serum neutralization test, as it was described in the manual, this assay has a sensitivity of 94.3 and 100 percent for sheep and goat and a specificity of 93.7 and 100 percent for sheep and goat, respectively. The plates were read in an automatic plate reader (Immunoskan Plus) at 450 nm and the results were expressed as optical density (OD). Samples with a corrected OD value below 0.25 were considered negative. The Rogan and Gladen's [15] correction of apparent prevalence was used for estimation of the true prevalence of seropositive samples. It was equated the true prevalence= (apparent prevalence +Sp-1)/ (Se+Sp-1) [15]. Demonstration of association between seroprevalence status and qualitative variables was carried out with the Chi-square and Fisher exact tests. Quantitative variables were subjected to two independent t-test. Significance was considered for alpha=5% (p<0.05) for a two- tailed test.

Table 1: Flock characteristics and potential risk factors of BDV infection in 13 small ruminant farms

Flock	Flock size	Flock System	Species (Sheep ,Goat or Mixed)	Density (Animal/m^2)	Female replacement origin	Abortion History	Exposure to other flocks
1	350	Resident	Mixed	2	Home bred	Yes	No
2	100	Nomadic	Mixed	-	Home bred	Yes	Yes
3	130	Nomadic	Mixed	-	Home bred	Yes	No
4	250	Nomadic	Mixed	-	Home bred	Yes	No
5	160	Resident	Mixed	3	Home bred/ purchased	Yes	Yes
6	80	Resident	Mixed	3.5	Home bred/purchased	Yes	Yes
7	300	Nomadic	Mixed	3	Home bred/ purchased	Yes	Yes
8	280	Resident	Sheep	7	Home bred	Yes	No
9	200	Resident	Mixed	2.5	Home bred/ purchased	Yes	Yes
10	200	Resident	Sheep	8	Home bred	Yes	No
11	120	Resident	Mixed	2.5	Home bred	Yes	No
12	120	Nomadic	Mixed	8	Home bred	Yes	Yes
13	200	Nomadic	Sheep	1.5	Home bred	Yes	No

Table 2: Number and percentage of BDV-seropositive animal in both female replacement procedures

Female replacement procedure	Number of Samples	Numbe of Seropositive	P value
Home bred	131	19	0.08
Home bred/ purchased	69	15	
Total	200	34	

Table 3: Number and percentage of BDV-seropositive in both species and relation to abortion history

Species	Abortion History	Number of Samples	Numbe of Seropositive	P value
Sheep	Yes	30	9	0.09
	No	66	10	
Goats	Yes	43	5	0.65
	No	54	8	
Total		200	34	

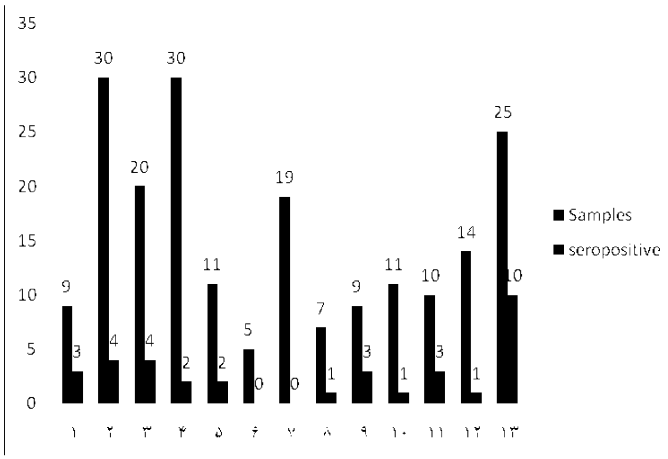

Fig. 1: Number of Sampled animal and seropositive animals to BDV in the 13 small ruminant flocks.

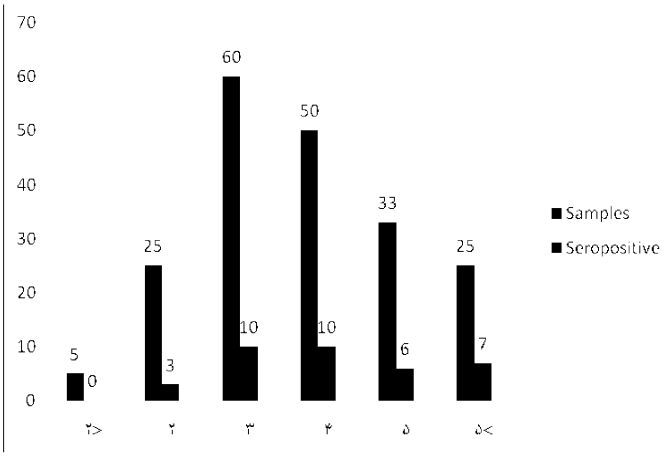

Fig. 2: Sampled animals and seropositive animals to BDV in Different age groups.

RESULTS

Antibodies to BDV were found in 34 (17%) of the tested sera (95% CI=11-23). Among 99 goat sera, 14% (95% CI=7-21) and 101 sheep sera, 20% (95% CI=12-28) were positive. However, the true BVD seroprevalence in sheep was 21.20 and 14.94% in goats. The difference in number of seropositive animals among different species was not significant (P<0.05). Antibodies could not be detected in two flocks. The percentage of seropositive animals within the flocks varies from 0 to 40% (Figure 1). Purchase of animals for female replacement in herds has

closed relation to seroprevalence of disease in this region in Fisher exact test (Table 2). Contact with other herds had no relation to animal prevalence in these herds (P=0.5). Among 19 seropositive and 77 seronegative ewe, 9(47.4%) and 21 (27.27%) had abortion history, respectively (Table 3). The results of this study indicated an increase of seroprevalence by increasing the age of the sampled animals, but the difference was significant (P<0.05) between sheep <2 years and sheep >2 years (Figure 2). In this study, density had no significant effect on seroprevalence of animal (P<0.05).

DISCUSSION

Surveys in the last years have shown that BDV infections occur worldwide and lead to significant losses in the small ruminant population [6-8, 16]. Antibody prevalence rates among sheep and goats ranged from 5 to 50% between countries and from region-to-region within countries [11]. In Northern Spain, serological surveys have shown 4-21% of the adult sheep and 10-93% of the sheep flocks to be pestivirus seropositive [17, 18]. A survey of antibodies to pestivirus in England and Wales found 10.8% of individual animals and 37.4% of flocks are seropositive [19]. The European work suggested an individual animal seroprevalence of 8.3% among Danish sheep [20]. Five of six French flocks showed positive serology [21], while an animal seroprevalence of 17.9% was reported. A pestivirus seroprevalence level of 4.5% among individual animals was documented in Norwegian sheep with 18% of sheep flocks showing exposure to the virus [22]. Seroprevalences of 25 and 66% to pestivirus were reported in two Irish flocks with recent histories of clinical border disease [23]. Serological investigations in Austria have shown a mean flock prevalence of 62.9% and a mean individual prevalence of 29.4% with marked regional differences [24]. Serological survey on prevalence of sheep border disease in some part of Iran was shown that ELISA total rate of infectivity came out to be 11.9%, while by SN test using border disease virus (isolated from Iran) this rate was 12.8% and by SN test, the rate of infection in all provinces was 13.5% [25]. Seroneutralization survey by NADL strain of Bovine viral diarrhea virus genotype 1 on small ruminants in Ahvaz (South West of Iran) was performed. The results indicated the overall seroprevanelces of 46.62% in sheep and 32.871% in goats [26].

The results of our study indicated 20 and 14% seropositive animal between sheep and goat, respectively and 84.61% flocks have at least one seropositive animal. True prevalence of sheep and goat were 21.20 and 14.94, respectively. Seventeen percent of total sampled animals were positive. As all sampled sheep were of breeding age, it was assumed that maternal antibodies no longer persisted and that antibody indicated direct exposure to pestivirus. The present study shows the prevalence of border virus infection in the sheep and goats of Shiraz suburb in Fars province of Iran. Pestivirus infection of sheep in other provinces of Iran has been previously reported by Keyvanfar *et al.* [25], but so far, there has not been any investigation on border disease virus infection of goat in Iran. Therefore, the present work is the first report of border disease virus infection of goat in Iran.

This is consistent with those by keyvanfar *et al.* [25] who found seroprevalence in small ruminant flocks in other regions between 3.2 and 18.7% [25]. The prevalence of bovine viral diarrhea in this region was 60.19% [27]. Results of these studies about small ruminant and cattle shown pestivirus circulated in cattle and small ruminant flocks of this region. Two herds of this study had no seropositive animal, which indicated this flocks had no PI animal or no contact with affected animals. Herd number 13 was higher seropositive animals in present investigation and had history of a meningocele lamb in 2010 spring. These findings may due to a PI animal and pestivirus presents in this herd. Several studies worldwide have reported prevalences of PI or viraemic sheep ranging from 0.3 to 20% in flocks with clinical BD [6, 18, 28]. One possible source of infection introduction in flocks may have been purchased animals. Purchased animal for female replacement may have an important role in increasing of serpositive animals, as shown in this study. Testing new animals for pestiviruses or keeping quarantine measures were not within farmer practices. Thus the seroprevalence of disease might be associated with the purchase of replacement sheep and goats.

Flock density in this study shown increasing density and prevalence are associated. Higher stocking densities and husbandry systems associated with more intensive sheep production may explain these higher levels of pestivirus seroconversion. Substantial regional variations in the use of particular sheep breeds and variable breed susceptibility to pestivirus may confound these management effects [29]. The results showed that BDV probably is related to ovine abortion in the region of course same as other parts of the world. With respects to the results obtained in this survey, it is suggested that for investigating the prevalence of PI animal in small ruminant flocks.The detected seroprevalence may underestimate pestivirus activity in infected flocks. The key for pestivirus control is the prompt identification and removal

of persistently infected (PI) individuals, combined with improved biosecurity measures to avoid reinfection. Further studies on the antigenic characterization and more extensive genome analysis of isolates will provide a deeper understanding of the etiology of pestivirus infections in ruminants in Iran.

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