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Seroepidemiology of bluetongue disease and risk factors in small ruminants of Shiraz suburb, Fars province, Iran

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Abstract. Bluetongue virus (BTV) is a member of the genus Orbivirus in the family Reoviridae which is transmitted by insects and could cause considerable damages in sheep and goat flocks, such as mortality, decreased production and fertility, medical costs and commercial limits for flocks and their biologic production. As no study has been conducted on small ruminants about this disease in Fars province of Iran, 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 an equal proportion of sheep and goats during the last three months of 2010. Some risk factors such as age, breed and abortion, exposure to other flocks, density and female replacement origin were reported in a question sheet. Totally, 73.5% of the samples were positive for presence of BTV antibody.The results showed that age and contact with other herds are influential risk factors on seroprevalence of the disease.

INTRODUCTION

Bluetongue virus (BTV) is the type specific to the genus Orbivirus within the Reoviridae family. Twenty four serotypes of the virus have been identified worldwide (Breard *et al.*, 2004). The virus is infectious but non-contagious, affecting domesticated and wild ruminants. The BTVs are transmitted between ruminant hosts entirely by the bites of certain species of Culicoides biting midges. There are also other possible means of transmission such as vertical transmission and oral transmission, although the last one is highly disputable (Mellor & Boorman, 1995; Gür, 2008). Among domestic animals, clinical disease occurs most often in sheep, and can result in significant morbidity (Pini, 1976).

Affected sheep may have erosions and ulceration on the mucous membranes, dyspnea or lameness from muscle necrosis and inflammation of the coronary band. Some strains of the virus can result in mortality rates as high as 70% in highly susceptible sheep (Breard *et al.*, 2004; Radostits *et al.*, 2007).

The BTV has been found in many parts of the world including Africa, Europe, the Middle East, Australia, the South Pacific, North and South America and parts of Asia. The virus is present in some regions without associated clinical disease (Breard *et al.*, 2004; Aradaib *et al.*, 2005; Shoorijeh *et al.*, 2008).

Various techniques have been used to detect antibodies against BTV. These include agar gel immunodiffusion (AGID), haemagglutination inhibition, complement fixation and ELISA, which are serogroup-specific and serum neutralization, which is serotype-specific. Although all these assays are available, only AGID and competitive-ELISA are recommended as prescribed tests for International Trade in the OIE Manual of Standards for Diagnostic Test and Vaccines (Breard *et al.*, 2004).

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

MATERIALS AND METHODS

The cross-sectional study was conducted on sheep and goat herds in the Shiraz suburb of Fars province, southern Iran. Two small ruminant production systems are described in this area. One is nomadic system; herds migrate in the spring and fall to find better feedstuffs and weather: another system is resident, owners feed herds with local grazing and complementary feedstuffs for the 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 an equal proportion of sheep and goats during the 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 study was 2490 animals. All of the farmers used natural breeding in their herds. Sheep and goats that had abortion history until one month before sampling 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 including contact with other herds, abortion history, density, race and age was discussed with the farmers and their practitioners, in a personal interview.

Blood samples were taken from the jugular vein into a plane 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 VP7 of BTV using a commercially available indirect ELISA (BT-Ab ELISA; IDVET) according to the manufacturer's instructions. The plates were read in an automatic plate reader (Immunoskan plus, USA) at 450 nm and the results were expressed as optical density (OD). According to the manufacturer's instructions, the mean OD of the negative control must be more than 0.7 and the mean OD of the positive control must be lower than 30 percent of the mean OD of the negative control. The competition percent was measured. If the percent was equal to or more than 40%, the sample was considered as negative, and lower than 40% was considered as positive. 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-tests. Significance was considered for alpha=5% ($P \leq 0.05$) for two-tailed test.

RESULTS

Antibodies to BTV were found in 147 (73.5%) of the tested sera (Table 1). The rate of positivity in goats and sheep was 74.2% and 72.9% respectively. Comparison of age in positive and negative groups showed that the age of the positive group is significantly more than the negative group ($P=0.012$). Also, contact with other herds had a relation to serum prevalence in these herds. Regarding abortion, the results showed that ewes with and without abortion history had 60% and 77.9% seropositivity, respectively. In goats, those with abortion history had 78.6 % and those without abortion history had 69.4% seropositivity (Table 2). Concerning the disease agent with abortion history, in Chi-square test, P value for sheep and goats was 0.07 and 0.32, respectively; which showed that this relation has closed with significancy for sheep, but not for goats. In the present study, density had no significant effect on seroprevalence of the disease ($P>0.05$).

Table 1. The result of ELISA for bluetongue antibodies in small ruminants of different areas of Fars province

Regions	Sheep	Positive	Negative	Goat	Positive	Negative	seropositivity
Darian	13	10	3	–	–	–	77%
Lar nomadic1	13	4	9	17	12	5	53%
Lar nomadic2	–	–	–	20	20	–	100%
Lar nomadic3	–	–	–	30	12	18	40%
Gerdkhun1	10	10	–	10	10	–	100%
Gerdkhun2	5	5	–	–	–	–	100%
Darab nomadic	8	4	4	11	10	1	73%
Cheshme bonab	8	5	3	–	–	–	62%
Kharameh	7	4	3	5	5	–	75%
Shahrak san'ati	16	16	–	–	–	–	100%
Kheirabad	13	13	–	–	–	–	100%
Marvdasht nomadic	14	7	7	–	–	–	50%
Total	107	78	29	93	69	24	73.5%

Table 2. The percentage of bluetongue antibody positive sheep and goats with and without abortion

Species				Abortion		Total
				No	Yes	
Sheep	ELISA	pos	Count	60	15	75
			% within ABORTION	77.9%	60.0%	73.5%
		neg	Count	17	10	27
			% within ABORTION	22.1%	40.0%	26.5%
	Total		Count	77	25	102
			% within ABORTION	100.0%	100.0%	100.0%
Goats	ELISA	pos	Count	34	33	67
			% within ABORTION	69.4%	78.6%	73.6%
		neg	Count	15	9	24
			% within ABORTION	30.6%	21.4%	26.4%
	Total		Count	49	42	91
			% within ABORTION	100.0%	100.0%	100.0%

DISCUSSION

Bluetongue is historically an African disease and although it has not been reported from all African countries, it is probably enzootic throughout the entire continent. Clinical recognition of the disease depends largely on the presence of highly susceptible European sheep breeds, which invariably act as indicator (Breard *et al.*, 2004; Erasmus & Potgieter, 2009). Surveys in recent years have shown that BTV infections occur worldwide and lead to significant losses in the small ruminant population (Radostits *et al.*, 2007; Shoorijeh *et al.*, 2008).

Serological studies showed that BTV infections were widespread in ruminants, that large ruminants had a higher seroprevalence than small ruminants and

confirmed the wider distribution across the country of serotypes that had been previously identified by virus isolation from the discrete groups of monitored sentinel cattle (Sendow *et al*., 1991). In Thailand, a serological study for BTV antibodies was carried out and demonstrated seroprevalences in the 60-75% range in indigenous cattle, sheep and goat (Apiwatnakorn *et al*., 1996). Seroprevalence rates up to 90% were detected in some regions of European Turkey (Taylor & Mellor, 1994).

Bluetongue was confirmed in herds located near the place where Belgium, the Netherlands and Germany share borders. The overall herd and true within-herd seroprevalences were estimated at 83.3 and 23.8% respectively (Franz *et al*., 2009).

The results of our study indicated 74.2 and 74.9% seropositive animals between goats and sheep, respectively. 73.5% of total sampled animals were positive. As all sampled sheep were of breeding age, it is assumed that maternal antibodies no longer persisted and that antibody indicated direct exposure to the Orbivirus. The present study showed the prevalence of bluetongue virus infection in the sheep and goats of Shiraz suburb in Fars province of Iran is considerable. A seroprevalence survey of bluetongue virus was reported in sheep flocks in West Azerbaijan, Iran. They reported that 172 of 184 flocks included BTV seropositive sheep (93.5%); serum evaluation using c-ELISA showed 34% seropositivity (Shoorijeh *et al*., 2008).

The results showed that the majority of animals in the area are infected with bluetongue virus, as there is no report of clinical signs, it is indicated that the disease is endemic (Radostits *et al*., 2007). Most probably, its epidemic in 1977 in Turkey caused the disease to become endemic in this area without any clinical signs (Taylor & Mellor, 1994). There could also be another reason that no clinical signs appear. As the disease is transmitted by insects and normally the insects can act as vectors for the other viruses simultaneously and cause some co-infections which could mask the clinical signs of each other as Taylor and Mellor explained during the epidemic of 1977 in Turkey which was accompanied by Akaban disease (Taylor & Mellor, 1994). By the way, it is assumed that there are some serotypes of the virus which cause disease without any typically clinical signs like the occurrence of disease in India in 2009 (Chand *et al*., 2009).

The distribution and intensity of infection in regions of the continents are determined by the climate, geography and altitude, as they affect the occurrence and activity of the Culicoides vectors and by the presence of susceptible mammalian hosts (Mellor & Boorman, 1995; Erasmus & Potgieter, 2009). The climate is a major risk factor as Culicoides require warmth and moisture for breeding and calm, warm humid weather for feeding (Purse *et al*., 2005). A cold winter or a dry summer can markedly reduce vector numbers and risk for the disease. Moisture may be in the form of rivers and streams or irrigation, but rainfall is the predominant influence and rainfall in the preceding months is a major determinant of infection. Optimal temperature is also essential and in endemic areas the temperature for survival of the adults and larvae requires temperatures sustained above a mean of 12.5°C for the cooler months and temperatures in the range of 18 to 30°C in the summer and autumn for optimum recruitment to adults and for optimal activity (Erasmus & Potgieter, 2009). According to our results the higher seroprevalence rate of BTV in Fars province and the surrounding regions (73.5%) compared with the southeast of Iran (Kerman) (6.57%) shows that there is a suitable climatic condition for survival of the adults and larvae of Culicoides vectors in this region; Mozaffari and Khalili related the low range of seropositivity to dry climate and high variations in temperatures of the southeast of Iran (Mozaffari & Khalili, 2012). The present study is furthur proof of this claim, as we conducted this study in 12 different regions of the province and then compared the results of these regions, the most highly positive regions, Kheirabad, Gerdkhun, Darian, Kharame with 100%,

100%, 77%, 75% seropositivity have a higher relative humidity and lower latitude than the other regions with lower seropositivity, this convinced us there is a relationship between suitable climatic conditions for vectors activity and BTV seropositivity.

The results of the present study indicated that the seroprevalence rates increase with the increasing of age in the studied herds, which is another reason for the disease to be endemic, because newborn lambs can be protected by maternal immunity. However, in a seroprevalence study in the southeast of Iran the results showed that seroprevalence rates decrease with the increase in age in sheep herds (Mozaffari & Khalili, 2012). Also, Taylor and Mellor reported that after BT epidemic in Turkey, the disease became endemic and probability of infection was much less in sheep up to 2 years old (Taylor & Mellor, 1994). In addition, according to the fact that Australian serotypes of the virus can affect sheep 3 years old or older, obviously there is a relationship between age of infected animals and serotype of the virus (Radostits *et al*., 2007).

Contact with other herds is another factor that affects the serum positive results. Probably the contact with the other herds increases the chance of exposure to the viral vectors which are living with the herds, because BTV is not transmited by direct contact with the infected animals.

It is reported that BTV can cause 25% abortion and 50% decrease in fertility in sheep (Toussaint *et al*., 2007). But in the present study there was no significant relation between seroprevalence rate and abortion history. It may be due to the infection with some serotypes causing subclinical infections or a long period endemic infection from the past.

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