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ORIGINAL ARTICLE

Bluetongue virus seropositivity and some risk factors affecting bluetongue virus infection in sheep flocks

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Abstract

Bluetongue (BT) is an insect-transmitted, viral, and non-contagious disease in several species of domestic and wild ruminants. Sheep is the most susceptible host for bluetongue virus (BTV) in ruminants. In current study, 556 serum samples of ewes were assessed by competitive ELISA to investigate the serum status of BTV antibodies and risk factors affecting its seroprevalence in sheep flocks of Hamedan province, west part of Iran. In total, 256 out of 556 (46%) samples were found seropositive against BTV in all examined counties with different prevalence (ranging from 23.9 to 85%). The mean of age in positive and negative groups (2.87 ± 0.83 and 2.94 ± 0.83, respectively) was not significantly different but the seroprevalence rates showed a reduction by increasing of age in sheep herds ($P < 0.05$). The analyzed data showed that 67.8% of abortions occurred in the second half of pregnancy and 25.0% of abortions happened in the first half of pregnancy period, and only 7.2% of lamb wastage occurred in the first day after labor. An association was found between seropositivity to BTV and abortion history, so that the seropositivity rates in ewes with abortion and non-abortion history were 41.7 and 4.3%, respectively ($df = 4$, $\chi^2 = 6.73$, $P = 0.0001$). The present study demonstrated the seroprevalence of BT in sheep is considerable in this region, so isolation and identification of involved serotypes of BTV and preparation of a precise control program with emphasis on vaccination and eradication of carriers are suggested for controlling of the BT.

Keywords Bluetongue · Abortion · Seropositivity · Sheep · Hamedan

Introduction

Bluetongue (BT), as a disease infecting the cattle and sheep, was described in the late eighteenth century for the first time (Mertens et al. 2009). BT is an insect-transmitted, non-contagious viral disease which affects many of domestic and wild ruminants. The infectious agent of BT is a pathogenic virus belonging to the *Orbivirus* genus (family: *Reoviridae*). Already 27 different serotypes of bluetongue virus (BTV) have been characterized and reported throughout the world (Schulz et al. 2016). BTV is transmitted from infected animals to the susceptible ruminants by the bite of a biological carrier, *Culicoides*. However, the mechanical transmission of the virus within a herd or between different herds by unsanitary tasks such as the use of contaminated needles and non-sterile surgical tools is also possible. Vertical and oral transmissions are the other likelihood routs of transmitting the disease to susceptible hosts, although the last one is in debate (Gür 2008; Mellor and Wittmann 2002).

BT is classified as "A" list of diseases by the World Organization for Animal Health (OIE) that has a considerable impact on the health and sanitary condition of livestock, because it can lead to considerable morbidity and mortality rates in infected animals and has the potential to cross the geographical boundaries of countries (World Organisation for Animal Health 2008). Among ruminants, sheep have the most sensitivity for BT infection. The clinical form of disease is rarely seen in bovine, but it seems that cattle are the main reservoir of the virus for other ruminants (Osburn 1994).

Erosions and ulcers of the mucous membranes in mouth and nose, lameness and hyperemia of the coronary band, weakness and depression due to high fever, and swelling of

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the face and tongue are the primary clinical signs in infected sheep (Maclachlan 2011; Maclachlan et al. 2009). Fatality rates as high as 70% may be observed in some susceptible sheep flocks following infection with some strains of the virus. There is no approved medical treatment for bluetongue, so the controlling and preventing strategies should be applied to decrease the cost of animal infection. Control of the BT spread due to the vector-borne nature of the disease, and global distribution of its vectors is less feasible. Prevention may be conducted by vaccination of animals with live modified virus vaccine and by controlling midge populations (with insecticides or by removing and control of reproductive grounds). However, the use of these methods is not entirely successful (Breard et al. 2004; Radostits and Done 2007).

Several diagnostic assays such as agar gel immunodiffusion (AGID), hemagglutination inhibition (HI), complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), and virus neutralization (VN) test have been used in laboratories to determine the level of antibodies against BTV. Among these cited techniques, competitive ELISA and VN are recommended methods in the OIE Manual of Diagnostic Tests and Vaccines (Breard et al. 2004).

The BTV initially has been considered as an endemic disease in Africa and Cyprus, but recently, it was identified in many regions of the world such as Australia, the USA, Israel, and European countries (Kyriakis et al. 2015). It has been reported that the presence of virus in some places of the world is not associated with clinical disease (Aradaib et al. 2005; Breard et al. 2004; Shoorijeh et al. 2010). In Iran, due to the favorable weather conditions, sheep and goat farming is of great importance. Hamedan, a province in west part of Iran, has been considered for livestock breeding, especially sheep, due to the suitable climate for agriculture and farming. So, this survey was conducted to investigate the serum status of antibodies against to BTV using competitive ELISA and risk factors (age, abortion history, and pregnancy trimester) affecting its seroprevalence in sheep flocks in Hamedan province.

Materials and methods

A cross-sectional study was carried out in Hamedan province from April to September of 2012. This province is located in the west of Iran between latitude 35° 58′ to 39° 47′ N and longitude 44° 14′ to 47° 19′ E (Fig. 1). A total number of 556 serum samples were collected from recently aborted and healthy ewes in the nine counties of Hamedan province. Animals over 6 months old were enrolled in this study. Tooth replacement of sampled sheep was used for age determination. Blood samples were collected from the jugular vein into a plane vacutainer tube and transferred in containers with ice to the Serological Laboratory of Veterinary Office of Hamedan. The samples were centrifuged immediately at 2000×*g* for 10 min at room temperature. Then, serum was separated and maintained at −20 °C before serological analysis.

ELISA assay

Serologically evaluation of blood sera for the presence of group-specific BTV antibody was performed by competitive ELISA (c-ELISA), using the c-ELISA IDEXX Bluetongue Competition® assay kit (IDEXX BT, Netherland) according

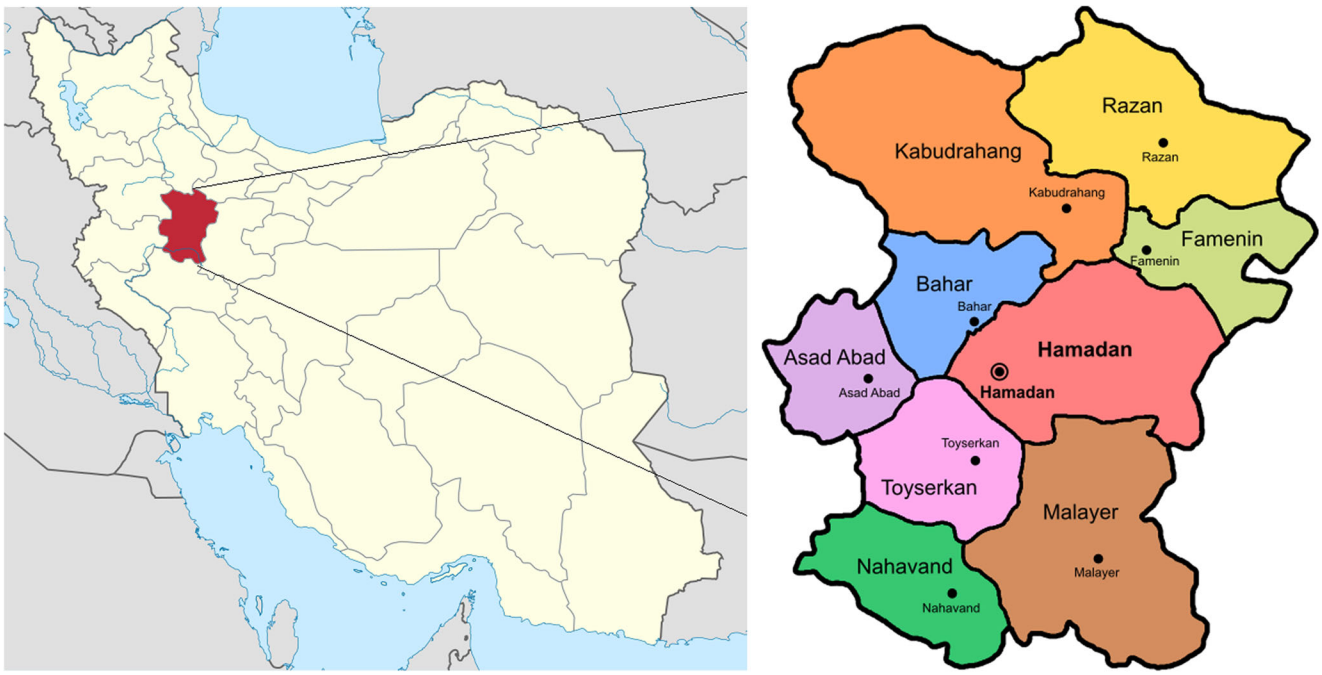

Fig. 1 Map of Iran showing the location of Hamedan province where the present study was carried out

Table 1 The ELISA result of bluetongue antibodies in serum samples of sheep from different regions of Hamedan province, west of Iran

Counties sampled	ELISA result		
	Positive	Negative	Seropositivity (%)
Famenin	36	15	70.6
Razan	35	69	33.7
Kaboudarahang	31	15	67.4
Bahar	22	70	23.9
Hamedan	45	62	42.1
Asadabad	17	3	85
Malayer	22	7	75.9
Nahavand	21	48	30.4
Touyserkan	27	11	71.1
Total	256	300	46.0

to the manufacturer's recommendations. The optical density of samples was read by an ELISA microplate reader (ELX 800, Bio-Tek Instruments Inc., VT, USA) at 450 nm.

Results are expressed as a percentage of negativity (PN) in comparison to the kit control and presented as positive (PN ≤ 70), doubtful (70 < PN < 80), or negative (PN ≥ 80) according to the manufacturer's recommendation.

Statistical analysis

Statistical analysis of data was performed using SAS software version 9.1. The chi-square (χ^2) and Fisher exact tests were used to evaluate the association between seroprevalence status, age, and abortion history. Differences were considered significant at $P \leq 0.05$.

Results

The seroprevalence results of the BT among sheep in Hamedan province are presented in Table 1. BTV antibodies were detected in 256 out of 556 (46%, 95% CI 42–50%) of samples (Table 1). Positive samples were found in all examined counties with different prevalence, ranging from 23.9 to 85%. The mean ± SD values of age (year) in positive and negative groups were 2.87 ± 0.83 and 2.94 ± 0.83, respectively, and no significant difference was observed ($P > 0.05$). The rate of seropositivity changed significantly in different age groups ($P < 0.05$). The seroprevalence rates decreased by increasing of age in sheep herds ($P < 0.05$) (Fig. 1). In our finding, 67.8% of abortions occurred in second half of pregnancy, 25.0% of abortions happened in first half of pregnancy period, and only 7.2% of lamb wastage occurred in the first day after labor. In respect of abortion history in the herd, the results showed that aborted ewes had 41.7% and those without abortion history had 4.3% seropositivity (Table 2 and Fig. 2). Concerning the correlation between the BTV seropositivity and abortion history, P value in chi-square test was equal to 0.0001 (Fig. 3).

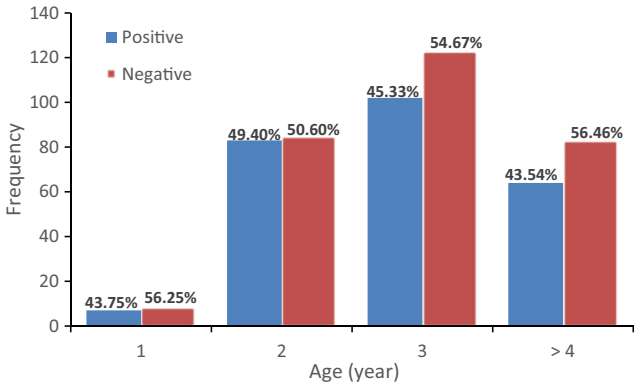

Fig. 2 Comparison of the positive and negative serum samples for BTV based on the age of tested sheep (df = 4, $\chi^2 = 7.63$, $P = 0.1062$)

Discussion

BTV can cause an infection in both domesticated as well as wild ruminant species specially sheep and transmitted between the susceptible hosts by hematophagous insects belong

Table 2 The percentage of bluetongue antibody in serum samples of sheep with history of abortion from different regions of Hamedan province

			Abortion history		
			Yes	No	Sum
ELISA result	Positive	Absolute count	232	24	256
		Relative frequency within this group	41.7%	4.3%	100%
		Relative frequency in all groups	90.63%	9.37%	46.0%
	Negative	Absolute count	128	172	300
		Relative frequency within this group	42.67%	57.33%	100%
		Relative frequency in all groups	23.0%	30.9$	53.9%
Sum		Total absolute count	360	196	556
		Total relative frequency	64.7%	35.2%	100%

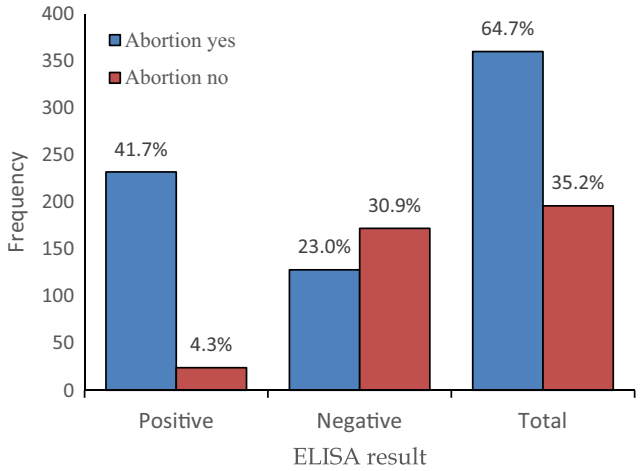

Fig. 3 Comparison of the ELISA result of BTV in sampled sheep with history of abortion in Hamedan province (df = 4, $\chi^2 = 6.73$, $P = 0.0001$)

to the genus *Culicoides*. For the first time, BT was identified in Merino sheep of South Africa in the late of eighteenth century (Gerdes 2004; Kyriakis et al. 2015). It has been reported in subsequent years as an infectious disease of domestic ruminants from many parts of the world such as America, Asia, Africa, Australia, and some European countries. Iran as a southwest country of Asia is bound by Iraq to the west and Turkey to the northwest that the seropositivity to BTV has been previously reported in those countries. BT is reported as an enzootic disease in Saudi Arabia, Syria, Egypt, Oman, Israel, Yemen, and Jordan; thus, these countries can be a potential sources of virus as the westward neighboring countries of Iran (Khezri and Azimi 2012a; b; Yousef et al. 2012). Seroprevalence rates of 68.6 and 48.8% for BTV have been reported in sheep from Afghanistan and Pakistan which are the two eastward neighboring countries of Iran, respectively (Akhtar et al. 1997; Ali et al. 2014). Therefore, the presence of BTV has been reported in the above countries as neighbors of Iran and makes it an important potential source of some strains of BTV that may transmit the disease to other adjacent countries (Oryan et al. 2014).

In this study, serum samples from sheep of Hamedan province were evaluated by competitive ELISA for the first time. The results indicated 46.0% (256 out of 556) of seropositivity in tested animals. According to the management strategies of sheep breeding in Iran, two points should be considered. Firstly, as all sampled sheep in this study were in reproductive age, the maternal antibodies are not available in their immune system yet and detected antibodies in analyzed serum samples are caused by direct exposure to the virus. Secondly, there is no vaccination program against BTV for ruminants in Iran, so antibodies detected against BTV have been derived from natural infection with the virus.

The presence of BTV in small ruminants has been assessed by many seroepidemiological studies throughout the world. Ventura et al. (2004) reported that the overall prevalence of BTV of sheep and goats in Albania was 4.4%. Woldemeskel et al. (2000) described that the prevalence of the bluetongue antibodies in sheep of Ethiopia was 46.67%. In Saudi Arabia, Yousef et al. (2012) reported the 54.4% prevalence rate for BT by competitive ELISA. In a recent study by Halder et al. (2016) for the assessment of BT seroprevalence in South Bengal, the overall seropositivity rates of 33.13 and 30.24% in sheep and goat was reported respectively as assessed by i-ELISA. They found that the predominant species of *Culicoides* as the vector of the disease were *Culicoides schultzei*, *Culicoides palpifer*, and *Culicoides definitus* during 2010–2013.

In a study by Khezri and Azimi (2013) in eight provinces of Iran between 2007 and 2008, the seroprevalence of BTV antibodies in sheep over the studied areas was estimated to be 34.93%, and the highest and lowest prevalence rates were found in West Azerbaijan (64.86%) and Qom (12.1%), respectively. They reported that the seroprevalence rates of BTV in Ilam and Kurdistan as two neighbors of Hamedan are 42.65 and 41.72%, respectively. A seroprevalence survey of bluetongue virus has been announced in sheep from the northwest of Iran. In this study, the BT seropositivity rates of 93.5 and 34% were reported in herd and animal's level,

Table 3 Annual data of climate factors (mean ± SD) of Hamedan province according to the report of Iran Meteorological Organization during 2012

Station points	Mean temperature (°C)	Total precipitation (mm)	Mean wind speed (m/s)
Hamedan	12.07	247.40	2.39
Asadabad	13.14	339.40	2.15
Touyserkan	13.39	398.20	1.83
Razan	13.75	291.90	3.48
Famenin	14.43	258.30	3.37
Malayer	13.52	286.90	3.29
Nahavand	13.99	323.50	2.35
Kaboudarahang	12.13	231.80	2.63
Ghahavand	14.68	210.90	2.31
Mean ± SD	13.46	287.59	2.64

respectively, using c-ELISA (Shoorijeh et al. 2010). Seroprevalence rates of 51.06% in Isfahan (400 out of 784 samples), 55.09% in Khuzestan (306 out of 556 samples), 73.5% (147 out of 200 samples), and 74.4% (610 out of 820 samples) in Fars provinces have been previously reported by competitive ELISA for the BTV antibodies (Mohammadi et al. 2012; Noaman et al. 2008; Noroozikia et al. 2014; Oryan et al. 2014). In a recent study by Noaman and Arzani (2016), the presence of antibodies against BTV in sheep from Chaharmahal-Va-Bakhtiari province has been evaluated using c-ELISA method. They concluded that 49.78% of the sampled animals (462/925) were seropositive for BTV.

The distribution and intensity of BT infection in different regions are influenced by some risk factors such as the weather condition, geographical situation, and altitude of the land, as these factors can change the activity of the *Culicoides* vectors. Moreover, the presence of susceptible mammalian hosts is essential for incidence of a new infection (Erasmus and Potgieter 2009; Kyriakis et al. 2015; Mellor et al. 2000). The climate has a serious effect on the distribution of the disease as *Culicoides* numbers and risk for the disease prevalence can markedly decrease after a cold or dry season. It has been described that calm, warm, and humid condition for feeding and warmth and moisture for breeding of these insects should be prepared (Purse et al. 2005).The temperature higher than the mean of 12.5 °C in the cooler months of year and temperatures between 20 and 30 °C in the summer and autumn are required for optimal activity (Erasmus and Potgieter 2009). Unfortunately, in the recent years, "climate change and global warming" provided the opportunity for biting midges to have an extended period of activity and expanded the time intervals for BTV transmission (Tweedle and Mellor 2002). This changes lead to widespread and numerous epidemics of BTV around the world. According to the annual data of Iran Meteorological Organization for Hamedan weather, climate change and occurrence of drought period in recent years provided an ambient temperature for the activity of vectors and transmission of BTV (Table 3) (www.irimo.ir).

According to our findings, the seroprevalence rate of BTV has a negative correlation with the increasing of sheep age. In a seroprevalence study presented by Mozaffari et al. (2012) conducted in the southeast of Iran, they reported that the rate of seropositive animals decreases with the increase in age in herds. In Turkey, Taylor and Mellor (1994) an epidemic incidence of BT concluded that the probability of infection in sheep up to 2 years old was much less as a result of endemic nature of the disease. However, the rate of seroprevalence decrease with the increase of age in studied sheep herds in a study by Mohammadi et al. (2012). They concluded that the disease is endemic because maternal immunity can protect newborn lambs against the BTV infection. Also, based on the presented findings that Australian serotypes of the BTV infect sheep with higher than 3 years old, inevitably, there is a correlation between serotype of the virus and age of infected animals (Radostits and Done 2007).

In conclusion, the prevalence of BTV infection in sheep of all counties in Hamedan province is noticeable. On the other hand, due to the presence of nomads and migratory livestock, there is no restriction on the movement of animals from one area to another within the country. Consequently, isolation and identification of different serotypes of bluetongue virus, a well-defined control strategy based on vaccination programs, vector eradication, and restriction on the transport of animals between different regions of the country, are required for preventing and controlling of the BT.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval During all stages of our study, all applicable international guidelines for the use and care of animals were followed. In addition, this article does not contain any studies with human participants or animals performed by any of the authors.

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