Comp Clin Pathol (2014) 23:1515–1523
DOI 10.1007/s00580-013-1815-4

ORIGINAL ARTICLE

Seroprevalence of bluetongue in sheep and goats in southern Iran with an overview of four decades of its epidemiological status in Iran

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Received: 18 June 2013 / Accepted: 11 September 2013 / Published online: 27 September 2013

Abstract Bluetongue virus (BTV) is the prototype of the genus *Orbivirus*, family *Reoviridae*. Bluetongue (BT) occurs throughout the temperate and tropical regions of the world, in an area that parallels the distribution of the competent vector, *Culicoides* spp. There is considerable genetic variability within the serogroup of BTV so that 26 serotypes have been recognized worldwide. A total of 1,010 blood samples from 820 sheep and 190 goats, with history of abortion and mucosal diseases, from 25 Counties of Fars Province, southern Iran were tested in a period of 1 year between 2010 and 2011, using competitive enzyme-linked immunosorbent assay (c-ELISA), for anti-bluetongue virus antibodies. A total of 772/1,010 (76.4 %) samples, 162/190 (85.3 %) goats and 610/820 (74.4 %) sheep, were found seropositive for BTV. The present study showed high seroprevalence of BT in goats and sheep of this area. The enzootic nature of BTV in southern areas of Iran is supported by climatic factors that favour the maintenance and recirculation of the virus in its vertebrate and non-vertebrate hosts. This investigation evaluates the present status of BT in southern Iran. It also has an overview on the previously confirmed serotypes of BT and demonstrates four decades prevalence of this disease in Iran.

Keywords Bluetongue · Sheep · Seropositive · Iran · Climatic factors · Fars

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Introduction

Bluetongue (BT) is an insect-borne disease caused by an *Orbivirus* of the family Reoviridae and affecting mainly domestic sheep breeds and occasionally cattle. It is transmitted by midges of a few select *Culicoides* species and its global distribution is largely defined by suitable climatological factors for these species. Bluetongue viruses (BTV) have been found in all continents excepting Antarctica but the disease is generally endemic in only the tropics and subtropics 34°S to 53°N (Hateley 2009; MacLachlan et al. 2009). So far, 24 BTV serotypes have been identified worldwide (Schwartz-Cornil et al. 2008) with the potential serotypes 25 and 26 recently isolated in Switzerland and Kuwait, respectively (Hofmann et al. 2008; Chaignat et al. 2009; Maan et al. 2011). BTV enters into the insect cells via the viral inner core VP7 protein and in mammalian cells via the external capsid VP2 haemagglutinin, which is the major determinant of BTV serotype and neutralization (Hawkes et al. 2000; Schwartz-Cornil et al. 2008).

Bluetongue viruses are amplified by ruminant hosts including cattle, sheep and goats. A total 1210 *Culicoides* species have been reported globally, but only 15 appear capable of transmitting BTV (Calistri et al. 2003). Although BTV is an arbovirus, it can occasionally be transmitted via seminal fluid and across the placenta (Parsonson 1990; Schwartz-Cornil et al. 2008).

The affected animals show marked depression, anorexia, pyrexia (up to 42 °C), copious salivation and development of an excessive serous or catarrhal nasal discharge which dries and forms crusts around the nose. Hyperemia, petechiation and swelling of the buccal mucosa, dental pads and tongue occur in the early stages of the disease. Later, the visible areas of hyperemia may become cyanotic and purplish-blue in color, and the appearance of the tongue gives rise to the popular description of the disease. At the end of the pyrexia

stage, the affected sheep may have coronitis, laminitis, and necrosis of striated muscles or paresis and, as a result, stand with an arched back and are reluctant to move. Torticollis, dermatitis and breaks in the wool may also develop (Brewer and MacLachlan 1994; Tweedle and Mellor 2002; Darpel et al. 2007; Elbers et al. 2008a, 2009; Kirschvink et al. 2009; Sperlova and Zendulkova 2011).

Infection in the pregnant ewes may lead to abortion, foetal mummification and the birth of weak lambs with potential congenital defects (hydrocephalus, cerebral cysts, retinal dysplasia, etc.) (Osburn 1994; MacLachlan et al. 2000; Tweedle and Mellor 2002; Saegerman et al. 2011). However, abortion has been considered to be secondary to the febrile illness affecting the ewes (Kirkland and Hawkes 2004).

The mortality rate is variable and in highly susceptible sheep it can be up to 70 %. Death may occur at any stage up to 1 month or more after the onset of clinical signs. Convalescence in surviving sheep is prolonged. Goats are less commonly, and less severely, affected than sheep. The pathogenesis in goat is similar to sheep but the clinical signs are milder (Sperlova and Zendulkova 2011).

The present study was designed to estimate the prevalence of BT in sheep and goats in southern Iran. The study has also an overview on the previously confirmed serotypes of BT and demonstrates a four-decades prevalence of this disease in Iran and clarifies favourable climatic conditions in establishment of the disease in Fars Province.

Materials and methods

This cross sectional study was carried out in Fars Province, southern part of Iran (Fig. 1).

A total of 1,010 blood samples were collected from aborted sheep and goats with the history of mucosal diseases in 25 cities of Fars Province, from December 2010 to March 2011.

Following the manufacturer's instructions, specific antibodies to the VP7 protein of the BTV in sera were detected by using a commercial ELISA kit (Pourquier, France). It is based on the competition between the samples to be tested and a monoclonal antibody which is coupled to the peroxidase. This monoclonal antibody is directed to the N-terminal part of the VP7 protein, a major core protein of the BTV (specific for the BT serogroup).

Record about monthly weather changes (temperature and precipitation) in the province from 22 synoptic points was recorded by the Fars Meteorological Organization, during March 2010 to March 2011.

The data were statistically analyzed by Chi square test and significance was considered for alpha=5 % ($P \leq 0.05$).

Results

Of the 1,010 sera tested, 772 were positive by BTV c-ELISA and the seropositive sheep and goats were evaluated 74.4 and 85.3 %, respectively (Table 1). All counties were positive, with different prevalence, ranging from 40 to 100 %, to BTV antibodies. The seroprevalence of BTV was significantly higher in goats than sheep [($P < 0.001$) $\chi 2 = 20.43$, $df = 1$].

Mean and standard deviation (SD) of the monthly weather records from 22 synoptic points were recorded and annually calculated (Table 2).

Discussion

The seroprevalence of 74.3 % BT in sheep and 85.26 % in goats, detected in the present study, demonstrates that the animals of this area are seriously at risk. The frequencies of positive goats were even significantly more than sheep ($P < 0.001$). The seroprevalence of BTV infection described in the present study (76.43 %) was markedly higher than most of the former studies in Iran (Table 3) and comparable to those which has been described amongst ruminants in East Azerbaijan Province (76.44 %) (Hasanpour et al. 2008), Khorasan Razavi (89.2 %) (Najarnezhad and Rajae 2013), northeastern areas of Fars (73.5 %) (Mohammadi et al. 2012) and Kerman, eastern Iran (67.7 %) (Mozaffari et al. 2012). The high seropositive rate in goats could indicate the importance of goats in transmission of this disease in this area. Higher prevalence of Bluetongue in goats than sheep has also been reported from Kerman, eastern Iran (Mozaffari et al. 2012).

Although occurrence of high prevalent abortion in the domestic small ruminants of this country has multifactorial etiologies, but the seroprevalence reported in the present study could represent the BT disease as a serious risk factor in predisposing the domestic and possibly wild ruminants to abortion (Mostaghni 1980). Afshar and Kayvanfar (1974) reported the existence of antibody against this virus by AGID test in Iran for the first time. Moakhar et al. (1988), Azimi et al. (2008) and Khezri and Azimi (2012a) performed serotype survey and reported the 3, 7, 20, 22, 9, 16 and 4 BTV serotypes at different areas of this country. The output of four-decades serological surveys of this disease at different areas of this country has been presented in Table 3.

The high prevalence of BT in small ruminants in southern Iran may reflect their involvement in the basic ecology of the virus. Shoorijeh et al. (2010) reported BTV antibodies in 34.7 % of sheep serum samples collected from West Azerbaijan, western Iran. Similar observations were made by Hasanpour et al. (2008) in East Azerbaijan Province.

Fars is one of the 30 provinces located in south of Iran (Fig. 2) between latitude 27° 02′ to 31° 42′ N and longitude 50° 42′ to 55° 36′ E. Twenty-nine counties are present in

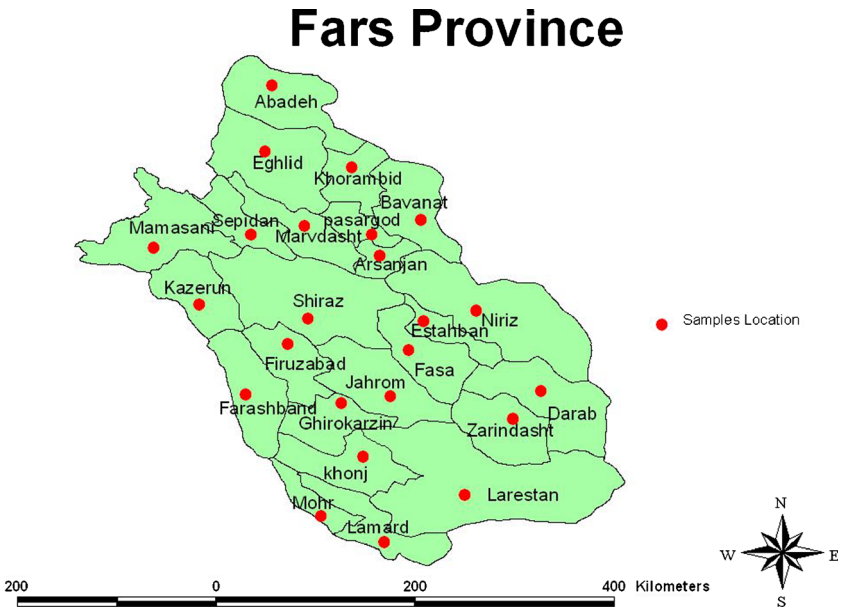

Fig. 1 Map of Fars Province showing the areas where the present study was conducted

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

Counties	Sheep		Goat		Total		%
	No.	Positive	No.	Positive	No.	Positive	Seropositive
Abadeh	28	22	15	11	43	33	76.74
Arsenjan	17	14	3	3	20	17	85
Bavanat	20	12	0	0	20	12	60
Darab	77	47	0	0	77	47	61.03
Eghlid	20	20	0	0	20	20	100
Estahban	20	16	0	0	20	16	80
Farashband	6	5	44	33	50	38	76
Fasa	25	22	25	23	50	45	90
Firuzabad	20	14	30	29	50	43	86
Ghirokarzin	80	68	0	0	80	68	85
Jahrom	42	27	0	0	42	27	64.28
Kazerun	50	38	0	0	50	38	76
Khonj	81	61	0	0	81	61	75.3
Khorambid	10	10	10	10	20	20	100
Lamerd	38	36	2	2	40	38	95
Larestan	52	48	0	0	52	48	92.3
Mamasani	12	8	31	29	43	37	86.04
Marvdasht	30	20	0	0	30	20	66.66
Mohr	10	4	0	0	10	4	40
Niriz	32	18	0	0	32	18	56.25
Pasargad	17	12	2	2	19	14	73.68
Sarvestan	20	10	0	0	20	10	50
Sepidan	20	18	0	0	20	18	90
Shiraz	13	12	28	20	41	32	78.04
Zarindasht	80	48	0	0	80	48	60
Total	820	610	190	162	1010	772	76.43

Table 2 Mean and standard deviation of annually climate factors from March 2010 to March 2011

Synoptic points	Mean temperature(°C)	Total precipitation(mm)
Abadeh	14.3	124.6
Arsenjan	18.6	183.5
Bavanat	13.5	138.8
Darab	22.5	280.7
Eghlid	13.1	311.1
Estahban	17.5	233
Farashband	21.8	213.7
Fasa	20	249
Firuzabad	20.9	327.2
Ghirokarzin	25.8	337.2
Jahrom	21.2	306.7
Kazerun	23.3	327.6
Khorambid	12.3	181.8
Lamerd	25.7	183.2
Larestan	24.1	169.6
Mamasani	21.7	436.4
Marvdasht	17.9	224.5
Niriz	19.6	178
Sepidan	15.4	652.3
Shiraz	18.4	239.4
Zarindasht	23.1	279.8
Mean ± SD	19.52±4.07	265.62±117.36

122,400-km^2 area of this province. There are three distinct climatic regions in Fars Province. Firstly, the mountainous area of the north and northwest areas have moderate cold winters and mild summers. Secondly, the central regions have relatively rainy mild winters and hot dry summers. The third region which is located in the south and southeast area has moderate winters with very hot summers. This province has significant populations of domestic ruminants, with 331,000 cattle and 8,644,000 sheep and goats, in 6,900 epidemiological units, which are known to be susceptible to BTV infection. These are either the native animals or belong to the nomadic people which migrate from other provinces including Iranian borders and stay for a season or less in this area. Therefore, it is possible that the BTV strains have been transmitted from the neighbouring countries and infected the animals of this area (Khezri and Azimi 2012a, b). Geography, climate and altitude of Fars Province are optimum for occurrence, survival and activity of the *Culicoides* vectors and the ability of biting midges to transmit BTV is markedly influenced by ambient temperature, humidity and total seasonal rainfall of this area (Mullens et al. 1995; Wellby et al. 1996; Mellor et al. 2000).

The virus can replicate in vectors at a temperature above 15 °C (Mellor et al. 2000) and the intensity of replication grows by increasing temperature (Van Dijk and Huismans 1982). The biting midges can fly over a maximum distance of 2 km, but because of their small size (1 to 3 mm), they can easily be carried on the wind and passively transport up to a distance of 700 km (Ducheyne et al. 2007). The recent 'global warming' has allowed for longer activity of biting midges and thus longer periods during which they are capable of BTV transmission (Tweedle and Mellor 2002). According to the annual data of Fars climate, occurrence of about one decade drought period changed the climate of Fars and provided an ambient temperature for vectors to transmit BTV (Table 2) (www.irimo.ir).

More than 2 % of the native cattle and 100 % of camel in Kerman, 38.38 % of sheep in Chaharmahal va Bakhtiari and Khuzestan, 46.77, 45.9 and 55.9 % of sheep from Kurdistan, Ilam and Azerbaijan and 53.4 and 49.2 sheep and goats in Isfahan have been found to be infected with BTV (Mahdavi et al. 2006; Mozaffari et al. 2010, 2012; Momtaz et al. 2011; Khezri and Azimi 2012a, b; Sadri 2012). Other studies have reported the seroprevalence of BTV antibodies from Khuzestan, Qum, Ardabil and Khorasan Razavi (Azimi et al. 2008; Khezri and Azimi 2012a, b; Najarnezhad and Rajae 2013). These reports established the fact that BTV infection is present in cattle, camel, sheep and goats in Iran.

Diagnosis of BT in suspected ruminants, in most instances, have been performed by clinical manifestations which is associated with major limitations. Firstly, clinical expression of BTV regarding strain and virus intensity, race of animals and environmental condition varies from peracute to subclinical manifestation. Secondly, symptoms of the disease in sheep can be mistaken with many other viral and even some nonviral diseases (Momtaz et al. 2011). Absence of BT in sheep does not necessarily imply absence of BTV or viral activity in a particular region or country. Sheep could therefore be regarded as merely an indicator of the presence of the disease. During the BTV epidemics in Europe in 2008, Williamson et al. (2008) considered clinical signs as the main tool in diagnosis of the disease. They showed low specificity of this method because some of the sheep that demonstrated signs of BT were infected with other diseases such as FMD, PPR, contagious ecthyma and haemonchosis (Tan et al. 2001; Elbers et al. 2008b). Occurrence of hemorrhage in the Tunica media of the pulmonary artery of sheep which has been regarded as pathognomonic for BTV infection (Worwa et al. 2010) also frequently occur in septicemic pasteurellosis and therefore has to be considered as differential diagnosis (Luja'n et al. 2005).

Seroprevalences of 48.8, 23.2 and 29.5 % BTV have previously been recorded in sheep in Pakistan, Iraq and Turkey, the neighbouring countries of Iran, respectively (Hafez et al. 1978; Akhtar et al. 1997; Gür 2008). Bluetongue is also enzootic in Jordan, Oman, Saudi Arabia, Syria, Israel, Yemen and Egypt, thus making these countries potential sources of virus for the Westward located regions (Khezri and Azimi

Table 3 Results of BTV in Ruminants in Iran between 1974 and 2013

Country	Provinces	BTV serotypes	Animal host	% Seropositive	References
Iran	[a]		Sheep	7.5	Afshar and Kayvanfar (1974)
Iran	[a]	3, 7, 20, 22	Sheep		Moakhar et al. (1988)
Iran	West Azerbaijan		Sheep and goat	63.1	Bokaie et al. (2009)
	Isfahan		Sheep Goat	53.37 49.19	No`man et al. (2006)
Iran	Kerman		Camel	100	Mahdavi et al. (2006)
Iran	East Azerbaijan		Sheep	76.44	Hasanpour et al. (2008)
Iran	Khuzestan, Fars, Ilam, Qum, Kurdistan,	9, 16, 4	Sheep		Azimi et al. (2008)
Iran	West Azerbaijan		Sheep	34.7	Shoorijeh et al. (2010)
Iran	Kerman		Cattle	2.13	Mozaffari et al. (2010)
Iran	[a]		Sheep	23.22	Azimi et al. (2011)
Iran	Chaharmahal, Khozestan, Isfahan		Sheep	30.38	Momtaz et al. (2011)
Iran	Kurdistan, Ilam	4	Sheep	46.77	Khezri and Azimi (2012a)
Iran	Kurdistan		Sheep	45.9	Khezri (2012)
Iran	Azerbaijan		Sheep	55.9	Sadri (2012)
Iran	Kerman		Goat	67.7	Mozaffari et al. (2012)
Iran	Kerman		Sheep	6.57	Mozaffari et al. (2012)
Iran	Fars		Sheep Goat	72.9 74.2	Mohammadi et al. (2012)
Iran	Khuzestan, Fars, Ilam, Kurdistan, Qum, Ardabil, E and W Azerbaijan		Sheep	34.93	Khezri (2012)
Iran	Khorasan Razavi		Sheep Goat	90 87.6	Najarnezhad and Rajae (2013)

[a] Unknown geographical region

Fig. 2 Satellite image of the study area of Fars Province (source: Google Map)

2012a, b). Presence of BTV in the above countries has been documented only relying on serological tests (Akhtar et al. 1997; Lundervold et al. 2003). Iran is located in the southeast of Europe and it makes it an important potential source of BTV strains and serotypes that may spread to adjacent countries or be a source to transmit the disease to European countries (Purse et al. 2005; Khezri and Azimi 2012a, b).

In endemic regions, the local ruminant breeds demonstrate resistance against the disease. Overt clinical signs are therefore usually only observed when susceptible breeds are imported into BT endemic regions or when the virus is introduced into immunologically naive flocks in regions where it is not normally encountered (Gibbs and Greiner 1994). The clinical presentation of BT also varies even amongst susceptible sheep, ranging from subclinical to acute disease that can lead to the death of infected animals. This variation in severity is influenced both by intrinsic differences in virulence between infecting strains as well as extrinsic host, vector and environmental factors such as breed, age, nutritional status, level of immunity, inoculum titer, temperature and UV radiation (Maclachlan 2004; Coetzee et al. 2012).

It has been shown that BT is more prevalent in the tropical and subtropical countries (such as Iran). In such areas generally, the disease appears subclinically and does not attract attention. In such circumstances, presence of the virus is mostly confirmed by serological evidences. However, in such foci, in spite of unrevealed disease manifestations, sudden occurrence of acute forms of the disease, in some instances, results in considerable death and economic loss (Nikolakaki et al. 2005). A large epidemic broke out on the Iberian peninsula, between 1956 and 1957, and subsequently bluetongue was also found in the Middle East, Asia and Southern European countries (Gibbs and Greiner 1994; Mellor and Wittmann 2002; MacLachlan 2004).

The 2006–2008 outbreak of BTV-8 in northern Europe represented an unprecedented step change in the epidemiology of the virus. This was the first time that BTV had been encountered in northern Europe in a region that was traditionally thought to be beyond the northern most limits where climatic conditions could sustain a bluetongue epidemic (Purse et al. 2005). Several features of the BTV-8 outbreak made it unusual when compared to outbreaks of BT occurring in other regions of the world. The BTV-8 strain was highly virulent and caused acute disease not only in sheep, but also in cattle and goats (Backx et al. 2007; Darpel et al. 2007). The BTV-8 strain also demonstrated the ability to cross the ruminants' placenta (De Clerq et al. 2008; Desmecht et al. 2008; Menzies et al. 2008; Vercauteren et al. 2008; Backx et al. 2009; Darpel et al. 2009; Saegerman et al. 2011; Santman-Berends et al. 2010; Van der Sluijs et al. 2011), a property that had previously been associated only with the vaccination of pregnant animals with the modified-live virus vaccine strains (Coetzee et al. 2012).

A new virus, similar to BTV, named *Toggenburg orbivirus*, and infecting goats has been discovered in Switzerland in early 2008. It is a so far unknown *Orbivirus* with low pathogenicity and a potential BTV serotype 25 (Hofmann et al. 2008; Chaignat et al. 2009).

Bluetongue can develop and spread when susceptible hosts, BTV and competent insect vectors are all present at the same time. Traditionally, the virus is present in a geographic band between the latitudes 40°N and 35°S where its vectors, certain species of biting midges, are living (Rodriguez-Sanchez et al. 2008; Vellema 2008; Wilson and Mellor 2009). In North America and China, the virus spread even further, up to 50°N (Mellor et al. 2000). Over the past 10 years, the global distribution of BTV has profoundly changed by spreading to the previously unaffected parts of the world such as most parts of Europe (MacLachlan et al. 2009; Worwa et al. 2010).

Vector species of *Culicoides* biting midge tend to breed in damp or wet soil enriched with fresh or composted dung and blood-feed opportunistically on large vertebrate hosts. Since appropriate breeding sites are very common around livestock holdings, *Culicoides* are particularly abundant at such sites and therefore feed predominantly upon domestic livestock, particularly cattle, horses, sheep and goats. They rapidly become much less abundant as distances from livestock holdings increase. *Culicoides* tend to be most active from about 1 h before sunset until one hour after sunrise. They are most active in the evening until about midnight, and then ease off with another peak of activity around sunrise (Tweedle and Mellor 2002).

Temperature can also affect the competence of the 'non-vector' *Culicoides* species. *Culicoides nubeculosus*, for example, is generally considered to be incapable of transmitting BTV due to a midgut infection barrier. However, exposure of the immatures to rearing temperatures close to their upper lethal limit (33–35 °C) can result in >10 % of the adults becoming competent to transmit BTV. It is likely that the integrity of the gut wall of some adults is damaged by the extreme rearing temperatures, thereby allowing virus particles to bypass the midgut barriers, enter the haemocoel and develop as in a normal vector. The increase in frequency and intensity of extremely warm days predicted to occur with climate change will enhance the chances of this phenomenon occurring in non-vector *Culicoides* species and hence could increase the number of BTV competent adults within populations (Gibbs and Greiner 1994; Tweedle and Mellor 2002).

The vectorial capacity of a *Culicoides* population, and hence the potential for virus transmission, is affected by (a) the number of adult midges in the population and (b) the proportion of adults capable of transmitting the virus, and is greatest when these factors are at their peak (Tweedle and Mellor 2002). Within favourable limits, the development rate of *Culicoides* from egg to adult is directly related to

temperature. Thus, increasing temperatures coupled with an extension in the developmental season may result in a greater number of generations, and therefore adults, per year (Johnson et al. 2006; Schwartz-Cornil et al. 2008).

In enzootic areas, BT usually appears in late autumn after long periods of quiescence (8–12 months), a phenomenon called overwintering (Takamatsu et al. 2003; White et al. 2004). In addition, the overwintering ability of adult *Culicoides* is likely to improve, as winters become both warmer and shorter. Improved overwintering success is also likely to increase the spring population input, which in turn could result in even larger populations during the summer (Mullens et al. 1995; Schwartz-Cornil et al. 2008). Changes in weather (temperature, precipitation, humidity and wind) and climate from global warming could produce wider distribution of vectors, resulting in increased prevalence of BTV at different parts of the world (Mellor and Wittmann 2002).

The incidence and geographical distribution of BTV infections are largely determined by the distribution of insect vectors and this can vary from year to year. Infection in sheep will usually be preceded by widespread infection of cattle and an increase in vector density. Cattle have an important epidemiological role as primary and amplifying hosts, and as ongoing sources of infection for vectors (Brewer and MacLachlan 1994; Schwartz-Cornil et al. 2008). Climatic conditions also have a significant impact on the transmission of BTV. For instance, insect survival is inversely related to temperature so that *Culicoides* insects survive for longer periods in cool temperatures. In contrast, higher ambient temperatures stimulate insect feeding and promote virogenesis of BTV in insects, both of which enhance virus transmission (Mullens et al. 1995).

Our findings demonstrated a high prevalence of BT antibodies in sheep and goats in Fars, providing serological evidence of exposure to BTV and the risk of establishment of BTV infection in all areas of Fars will be influenced by the following situations:

(a) The population density of animals, particularly cattle
(b) The level of susceptibility of the animal population to BTV infection
(c) The abundance of local competent *Culicoides* vectors
(d) No animal movement restrictions
(e) No vaccination program
(f) No early warning and eradication program

In conclusion, this study demonstrated that increase in the susceptible population, along with favourable climatic conditions, appears to have led to the establishment of BT in Fars Province and history of abortion in small ruminants can be remarkable as a sign in BTV infection. BTV genetic and phenotypic diversity are recommended for further research.

Acknowledgements The authors gratefully acknowledge the staff of Fars Veterinary networks for assistance in sampling and grateful thanks are extended to Fars Veterinary administration for contributed support.

Conflict of interest The authors know of no conflict of interest.

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