Original Article

Seroprevalence of bluetongue virus infection and associated risk factors in domestic ruminants in the south of Iran

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Abstract

A cross-sectional study was designed and 3,872 blood samples were collected from apparently healthy sheep, goats and cows in Fars province, the south of Iran. The sera were screened for detection of bluetongue virus (BTV) antibodies using a commercially competitive enzyme-linked immunosorbent assay kit. Overall prevalence rate of BTV antibodies among the domestic ruminants was 57.64% (70.93%, 55.70% and 19.77% for sheep, goat and cow, respectively). Associations between each independent variable, including ruminant type, age, sex, region, farming system and breed, and BTV infection were statistically significant ($p<0.05$). Females were 1.32 times more likely to exhibit seropositivity. Odds ratio for BTV infection was higher in older animals and traditional farming. The results revealed a wide spread of BTV infection in the domestic ruminants, especially in sheep and goat. Further studies of the distribution of *Culicoides* vectors in the region, virus isolation and genome sequencing of the isolated viruses are suggested.

Keywords: bluetongue virus, Fars province, Iran, risk factors, seroprevalence

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Introduction

Bluetongue (BT) was first described in Iran in 1974 (Afshar and Kayvanfar, 1974). It is a viral infectious disease but non-contagious in both domestic and wild ruminants. Bluetongue virus (BTV) belongs to the genus *Orbivirus* in the family *Reoviridae*. It is transmitted by different species of *Culicoides* biting midges and maintained in nature in a vector-ruminant host cycle. Among 1,400 species of *Culicoides* midges only 20 species are known to be involved in the transmission of bluetongue disease (Pandrangi, 2013). Initially, 24 serotypes of BTV were recognized worldwide, but recently 25, 26 and 27 serotypes have been discovered in goat from Switzerland, Kuwait and France, respectively (Hofmann et al., 2008; Maan et al., 2011; Schwartz-Cornil et al., 2008; Schulz et al., 2016). Sheep are often severely affected by the disease while cattle are considered to be the main reservoir host (Pfannenstiel et al., 2015). Bluetongue is one of the major causes of economic loss to farmers and it has been included in the list of notifiable diseases by the World Organization for Animal Health (Weaver and Reisen, 2010). Overall loss of productivity, weight loss, wool break and mortality along with a huge loss as a result of a ban on the export of live animals are the main causes of economic loss (Wilson et al., 2009).

Bluetongue is endemic in the tropical and sub-tropical regions and has spread between the latitudes of 40°N and 35°S, also in certain regions of North America and China up to 50°N (Gibbs and Greiner, 1994). Studies have been continuously conducted worldwide to better understand the epidemiology of BT. The published serologic information of BTV was reviewed in the Caribbean and South and Central America (Legisa et al., 2014) and South Africa (Coetzee et al., 2012). Several researchers have studied the seroprevalence of BTV in different regions of Iran and reported high seropositivity in small ruminants in contrast to cow (Mohammadi et al., 2012; Mozaffari et al., 2012; Mozaffari et al., 2014; Najarnezhad and Rajae, 2013; Noaman et al., 2008; Noaman et al., 2013; Oryan et al., 2014; Sabaghan et al., 2014). Various methods to detect BTV-specific antibodies have been developed like agar gel immune-diffusion, hemagglutination-inhibition, complement fixation, enzyme-linked immunosorbent assay (ELISA) either blocking ELISA or competitive ELISA (C-ELISA) and serum neutralization test (Afshar, 1994). The C-ELISA is a rapid method permitting determination of serum or plasma antibody as early as the 6th post-infection day and is recommended as a prescribed test for international trade (OIE, 2011; Sperlova and Zendulkova, 2011). Goat, sheep and cow populations of Fars province are approximately 4.35, 3.83 and 0.38 million heads, which rank first, second and fifth of the animal population in Iran, respectively. They produce 497 million liters of milk and 61 million kg of red meat annually and play an important role in the economy of farmers in this region (Agriculture Statistics of Iran, 2013). No report has been published on BTV seroprevalence in cattle and associated risk factors in the south of Iran. The main objective of this study was to evaluate the seroprevalence of BTV in domestic ruminants and to identify potential risk factors associated with BTV infection in Fars province, Iran.

Materials and Methods

Study design and area: A cross-sectional seroprevalence study was conducted by collecting a total of 3,872 blood samples from sheep, goat and cow in different areas of Fars province (in the south of Iran). This province is located between latitude 27°3' to 31°40'N and longitude 50°36' to 55°35'E in an area about 133000 km^2 with mean annual rainfall of about 230 mm (Figure 1). Cities of the province were classified into two groups based on the mean annual rainfall (above and less than 300 mm). Three epidemiological units were randomly selected in each group according to the Geographic Information System of Iran Veterinary Organization. The blood samples were taken from all animals in each region from January 2011 to March 2011.

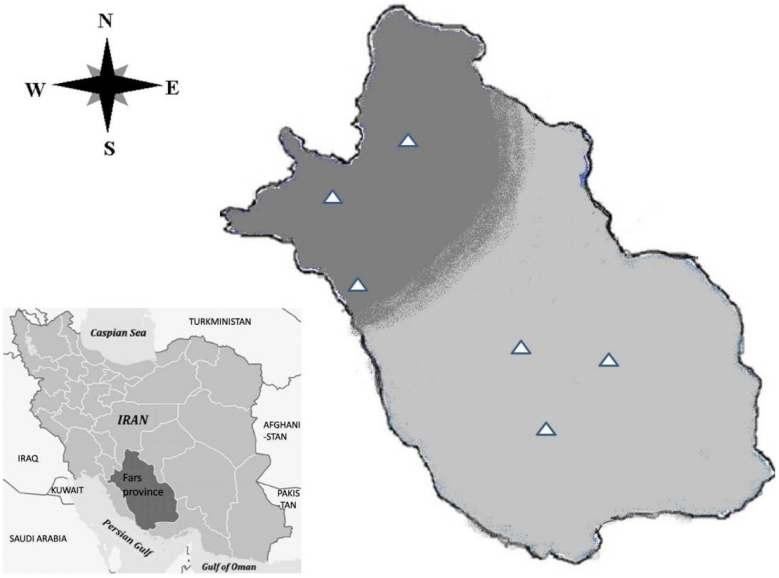

Figure 1 Geographical location of Fars province in Iran. In the Fars province map, the triangles indicate the locations of epidemiologic unites and the light and dark areas represent regions with ≤ 300 ml or > 300 ml annual precipitation, respectively.

Sample collection and preparation: The study protocol was approved by the research board of Razi Vaccine and Serum Research Institute, Karaj, Iran. Objectives and methods of the study were described to animal owners. The blood samples were collected with no or minimal pain to the animals by experienced veterinarians following proper physical restraint of animals to ensure both personnel and animal safety. The blood was collected from the jugular vein (5 ml) into sterile vacuum tubes (VACUETTE®, Greiner Bio-One GmbH, Kremsmünster, Austria). The sera were separated by centrifugation (10 min) at 3000 rpm and stored at -20◦C until analysis. Data including age (newborn, under 6 months; young, between 6 months and 2 years; and old, above 2 years), sex (male or female), breed (indigenous or exotic) and history of abortion were obtained from the animal owners by a questionnaire.

Detection of antibodies: Specific antibodies to the VP7 protein of BTV in the sera were searched using a commercial C-ELISA kit (Pourquier®, Montpellier, France). Relative specificity of this kit was recorded 100% using a panel of sera originating from healthy cattle never vaccinated or exposed to BTV. The relative sensitivity of ELISA kit was also high on the basis of testing either BTV-infected (100%) or vaccinated (82.2%) cattle (Niedbalski, 2011). The basis of the test was to detect antibodies against any serotype of BTV. The wells in the microplate strips were coated with a recombinant VP7 protein and filled with 80 µL of dilution buffer provided by the manufacturer. One positive and two negative controls were included in each test and other wells were filled with 20 µL of serum and incubated. Specific antibodies to VP7 were proportionally bound by shaking the plate gently and incubating at room temperature for 45 min in the dark. Anti-VP7 conjugated to horseradish peroxidase (100 µL) was added to the wells and the incubation step was repeated. The wells were washed with 300 µL of washing buffer three times and the liquid was aspirated after each wash. The conjugate bound to corresponding epitopes on the microplate in the absence of VP7 antibody in the sample and unbound conjugate was washed away when VP7 antibodies existed. Then, 100 µL of Tetramethylbenzidine liquid substrate was added to the wells and mixed gently by shaking the plate manually and incubated at room temperature for 10 min in a dark place. The liquid was detected to contain horseradish peroxidase activity and developed a blue color. Finally, 100 µL of 1 N sulfuric acid stop solution was added to the wells, which led to a color change from blue to yellow. Absorbance was measured at 450 nm in an Elisa plate reader (Bio Tek, Vermont, USA). Results were expressed as a percentage of the S/N ratio, calculated as S/N% = 100 × sample absorbance/negative control mean absorbance. According to the manufacturer's specifications, a sample was considered to be positive for bluetongue virus antibody if its S/N% value was less than or equal to 70% (Noaman et al., 2013).

Statistical analysis: All statistical analyses were carried out in SPSS for Windows 16.0.0 (SPSS Inc., 2007). Data were analyzed descriptively in the first step. Relationship between the seroprevalence of BTV and animal type, age, sex, region, farming system, breed and history of abortion was assessed by Chi square ($\chi 2$) test. P-values < 0.05 were considered statistically significant. Logistic regression analysis was used to calculate odds ratios and 95% confidence interval (CI) for risk factors of BTV.

Results

The test was positive in 2,232 of the 3,872 animals and the overall prevalence rate of BTV antibodies among the domestic ruminants in Fars province was 57.64%. Results of the univariate analysis using the Chi-square test indicated that all associations between the independent variables, including ruminant type, age, sex, region, farming system and breed, and BTV infection were statistically significant ($p<0.05$) (Table 1).

The logistic regression model was statistically significant, $\chi 2_{(7)} = 51.50$, $p<0.001$. The model explained 34.6% (Nagelkerke R^2) of the variance in BTV infection and correctly classified 72.5% of the cases. The odds ratios for risk factors are shown in Table 2. The effect of animal breed on the rate of BTV was not significant in the final model. Females were 1.32 times more likely to exhibit seropositivity. The odds of BTV infection were higher in older animals and traditional farming when compared to those estimated for younger animals and industrial farms.

Discussion

A quick and simple method for evaluating the exposure to BTV in animal with high sensitivity (100%) and specifity (98%) is cELISA (Khezri and Azimi, 2013; Noaman et al., 2013). This method has been used in several cross-sectional studies in different regions of Iran and results showed high prevalence of BTV infection in sheep and goats unlike in cattle. The seroprevalence of BTV in sheep and goats in the present study was lower than that reported in the south and northeast of Iran (Mohammadi et al., 2012; Najarnezhad and Rajae, 2013; Oryan et al., 2014), but higher than that obtained in Isfahan province, center of Iran (Noaman et al., 2008). Antibodies against BTV was detected in 70.93% of sheep in the present study, which are lower than those detected in Kohgiluyeh and Boyer-Ahmad province, southwest of Iran (77.48%) (Sabaghan et al., 2014) but higher than those detected in West Azerbaijan (34.70%) and the northwest (33.75%) and west (46.10%) provinces of Iran, respectively (Khezri and Azimi, 2013; Shoorijeh et al., 2010). The seroprevalence of BTV in cattle in the southeastern and center of Iran was 2.13% and 2.69%, respectively, which is low when compared to our result (19.77%) (Mozaffari et al., 2012; Noaman et al., 2013). Contrary to our findings, high BTV seroprevalence in Turkey (88%), Kenya (94.2%) and Taiwan (32.7%) was detected in cattle (Gur, 2008; Lee et al., 2010; Toye et al., 2013). These discrepancies may be explained by variations in climate, population of *Culicoides* and ruminant husbandry systems.

BTV infection of ruminants has been well documented in tropical and subtropical regions of the

world such as the Middle East. Iran is located in the Middle East and four different climates prevail in the regions of the country simultaneously during the year, therefore it is usually known as the country with four seasons at a time. For example, when the weather is cold and snowy in the northwest and west, people in the south and southeast of Iran experience warm and semi-dry weather. These variations can influence the density of vector population. Vector presence and ability to transmit BTV are related to climatic factors including temperature, precipitation, humidity and wind conditions (Mellor and Wittmann, 2002). Cold weather as well as dry weather can significantly reduce vector numbers, because *Culicoides* require warm and humid conditions for feeding and breeding (Purse et al., 2005). Hot and dry climatic renders environmental conditions unfavorable for the activity and maintenance of the life cycle of the insect vector (Khair et al., 2014). Ambient temperature, humidity and total seasonal rainfall of Fars province are optimum for proliferation, propagation and activity of the *Culicoides* vectors and consequent transmission of BTV (Oryan et al., 2014). There are two systems of sheep and goat husbandry in Fars province, the extensive system, which is practiced in rural areas with stationary grazing, and the nomadic system, in which a herd moves among provinces in definite times. Animals graze on semi-arid rangeland pastures in both systems. Instead, most cattle are kept in intensive system and fed in barn in this area. Although it is widely accepted that wind can assist vectors in dispersing over a great distance, restriction of movement of animals is effective at reducing the spread of BT (Turner et al., 2012). Therefore, graze based management can be a probable cause for the higher prevalence of disease in sheep and goats in comparison to cattle in this study. Animals with age under six months had lower seroprevalence rates than older animals, which is in accord with other reports in cows (Adam et al., 2014; Noaman et al., 2013) and sheep (Sabaghan et al., 2014). This is probably a reflection of loss of colostral antibodies, increased susceptibility with age, elevation of a previously undetected antibody response, greater exposure to the vector or larger total body surface area in older in comparison to younger animals (Uhaa et al., 1990). However, this association between BTV infection and age has not been reported in sheep in some studies conducted in Pakistan and Nepal (Akhtar et al., 1997; Gaire et al., 2014). The seroprevalence in females was significantly higher than in males. This finding is in agreement with findings of a number of authors in Iran and in contrast to the results of studies in sheep and goats in Uttar Pradesh, India and cattle in Western Sudan (Bitew et al., 2013; Khair et al., 2014; Sabaghan et al., 2014). A significant difference in BTV seroprevalence was seen between animals kept in industrial and non-industrial farms. It can be explained by the low hygienic conditions in non-industrial farms which provide a suitable environment (damp or wet soil enriched with fresh or composted dung) for sexual and biting activity of the vector. Relationship between BTV infection and farming system was not shown in cattle in central Iran (Noaman et al., 2013). Breed was another factor which significantly affected the seroprevalence of BTV, but this might be due to the very higher number of samples collected from indigenous breeds than exotic breeds. Although it has been reported that a history of abortion in sheep is positively associated with seropositivity to BTV (Akhtar et al., 1997; Gaire et al., 2014), this association was not detected in the present study.

Table 1 Relationship between potential risk factors and BTV infection

Risk factors	Number of tested animals	Number of positive animals	Seroprevalence (%)	$\chi 2$	P-value
Animal				437.9	0.001
Sheep	1782	1264	70.93		
Goat	1569	874	55.70		
Cattle	521	103	19.77		
Age				166.3	0.001
Under 6 months	1105	461	41.72		
6 months-2 years	1435	912	63.55		
Above 2 years	1332	868	65.17		
Sex				73.44	0.001
Female	2977	1834	61.61		
Male	895	407	45.47		
Region (based on precipitation)				102.8	0.001
≤ 300 ml	2650	1389	52.42		
> 300 ml	1222	852	69.72		
Farming system				573	0.001
Traditional	3474	2234	64.31		
Industrial	241	5	2.07		
Semi-industrial	157	2	1.27		
Breed				549.6	0.001
Indigenous	3461	2225	64.29		
Exotic	411	16	3.89		
History of abortion				1.3	2.54
No	3858	2235	57.93		
Yes	14	6	42.86		

Table 2 Odds ratios (OR) of risk factors for BTV infection

Risk factors	OR	95.0% CI	P-value
Animal			
Sheep	1		
Goat	0.429	0.367-0.502	0.001
Cattle	0.294	0.186-0.467	0.001
Age			
Under 6 months	1		
6 months-2 years	4.681	3.794-5.776	0.001
Above 2 years	1.732	1.732-1.423	0.001
Sex			
Female	1		
Male	0.682	0.569-0.816	0.001
Region (based on precipitation)			
≤ 300 ml	1		
> 300 ml	1.676	1.418-1.981	0.001
Farming system			
Traditional	1		
Industrial	0.003	0.001-0.015	0.001
Semi-industrial	1.639	0.313-8.571	0.558

This study revealed the wide spread BTV infection in domestic ruminants, especially in sheep and goat in Fars province, the south of Iran. Small ruminant husbandry is based on movement and grazing on rangeland in Fars province. It implies that these animals can serve as a potential threat for other domestic ruminants in the region and the whole country. Vaccination against BTV has not been practiced in Iran and antibodies detected in ruminants implied natural exposure to BTV infection. Further studies of the distribution of *Culicoides* vectors in the region, virus isolation and genome sequencing of the isolated viruses are suggested. Vector control and farming education about BT management are also recommended.

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บทคัดย่อ

ความชุกทางซีรัมวิทยาของการติดเชื้อไวรัส bluetongue และความสัมพันธ์กับปัจจัยเสี่ยงในสัตว์เคี้ยวเอื้องในตอนใต้ของประเทศอิหร่าน

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การศึกษาระบาดวิทยาแบบ cross-sectional ในตัวอย่างเลือดจำนวน 3,872 ตัวอย่างจากแกะ แพะ และวัวสุขภาพดี ในจังหวัดฟาร์ส ซึ่งตั้งอยู่ทางตอนใต้ของประเทศอิหร่าน โดยตรวจหาแอนติบอดีต่อเชื้อไวรัส bluetongue (BTV) ด้วยชุดทดสอบอิไลซ่า ผลการศึกษาพบว่า ความชุกของแอนติบอดีต่อ BTV ในสัตว์คิดเป็น 57.64% (70.93% 55.70% และ 19.77% ในแกะ แพะ และวัว ตามลำดับ) และพบว่า ตัวแปรอิสระ ได้แก่ ชนิดสัตว์เคี้ยวเอื้อง อายุ เพศ ภูมิภาค ระบบการเลี้ยง และสายพันธุ์ มีความสัมพันธ์กับการติดเชื้อ BTV อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) โดยในสัตว์เพศเมียพบ seropositivity สูงกว่าในสัตว์เพศผู้ 1.32 เท่า นอกจากนี้ odds ratio การติดเชื้อ BTV มีค่าสูงในสัตว์สูงอายุและการเลี้ยงแบบพื้นเมือง ผลการศึกษาครั้งนี้แสดงถึงการติดเชื้อ BTV อย่างกว้างขวางในสัตว์เคี้ยวเอื้อง โดยเฉพาะอย่างยิ่งในแกะและแพะ ในอนาคตควรมีการศึกษาการกระจายของพาหะ Culicoides ในภูมิภาค การเพาะแยกเชื้อไวรัส และจีโนมของเชื้อไวรัส

คำสำคัญ: ไวรัส bluetongue จังหวัดฟาร์ส อิหร่าน ปัจจัยเสี่ยง ความชุกทางซีรัมวิทยา

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