Journal of Scientific Research & Reports
3(6): 787-798, 2014; Article no. JSRR.2014.003

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Investigation of Bluetongue in Sheep in Western Iran with an Overview of Infection Since 1972

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Authors' contributions

This work was carried out in collaboration between authors. Authors MB and MK designed the study. Author MK carried out the experiments, results analysis and drafted the manuscript. Author MB led the project, designed the study and revised the results and manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 19th November 2013
Accepted 21st January 2014
Published 29th January 2014

ABSTRACT

Aim: Bluetongue virus (BTV) is an economically important O*rbivirus* of the *Reoviridae* family, causes a haemorrhagic disease mainly in sheep and occasionally in cattle and some species of deer. The aim of this study was to describe the seroprevalence rate of bluetongue virus (BTV) in sheep in Kurdistan province, west of Iran. Also the history and epizootiology of bluetongue (BT) infection in Iran are reviewed.
Study Design: method depends on Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA).
Place and Duration of Study: Departments of Veterinary, Agricultural and Natural Resources Research Center and Departments of Animal Virus Disease, Razi Vaccine and Serum Research Institute, in west of Iran during 2011-2012.
Methodology: A total of 297 field sera collected from Healthy sheep with no symptoms in western Iran were screened for the present of group-specific BTV antibodies by competitive ELISA (c-ELISA).
Results: The overall BTV antibodies prevalence was 42.42% in sheep (at 95% confidence level). Also a result was showed a significant increase in seroprevalence BT

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antibodies with classes of age.
Conclusion: This investigation evaluates the present status of BT in western Iran. As well as the outbreak of BT in farm animals in the country was not recorded. A serological survey has indicated the presence of bluetongue virus (BTV) antibodies in sheep, goats, cattle and other farm animals in several states in Iran. However, clinical BT has not been observed in farm animals to date. BTV has not been isolated from *Culicoides midges*, although virus serotypes 3, 4, 9, 16, 20 and 22 were isolated in Iran.

Keywords: Antibodies; bluetongue virus; C-ELISA; Iran; seroprevalence; sheep.

1. INTRODUCTION

Bluetongue virus (BTV) is classified as a species or serogroup in the *Orbivirus* genus in the family *Reoviridae*, one of 22 recognized species in the genus that also includes epizootic hemorrhagic disease virus (EHDV), Equine encephalosis virus (EEV) and African horse sickness virus (AHSV) [1]. Within species, individual members are differentiated on the basis of neutralization tests, and the vertebrate hosts for BTV include both domestic and wild ruminants, such as sheep, goats, cattle, buffaloes, deer, most species of African antelope and other Artiodactyl such as camels. Although antibodies to BTV and in some cases virus antigen and/or live virus has been demonstrated in some carnivores, felids, black and white rhinoceroses and elephants, the role of non-ruminant species in the disease in the wild is not known. The outcome of infection ranges from in apparent in the vast majority of infected animals, especially wild Africa ruminants and cattle, to fatal in a proportion of infected sheep, goats, deer and some wild ruminants [2]. In cattle and goats, infection occur mostly asymptomatically [3]. Cattle might act as a reservoir of infection, although some authors have indicated that BTV does not persist in naturally infected cattle [4]. There are 24 distinct BTV serotypes and recently Toggenburg orbivirus (TOV) is proposed to be a 25th serotype [5] and complete genome characterization of 26th BTV serotype from Kuwait was reported [6]. It is a notifiable disease of the World Organization for Animal Health (Office of international epizooties: OIE) due to its economic impact [7], Also BT is a multiple species disease to the OIE [8]. Kurdistan is located in west of Iran. There are two distinct climatic regions in Kurdistan Province. Firstly, the mountainous area at the west area has moderate cold winters and mild summers. Secondly, the plains region at the east has relatively rainy mild winters and dry summers. This province has a common border with neighboring countries and local animals that live in the area frequently cross the boundaries. However, the cities in this province have similar livestock. About 2.5% of the livestock are sheep, up to 2.4% of the goat and 0.3% of the cattle population are kept in the province; this means that any potential disease that infects the livestock could threaten the human population, and will therefore have a great impact on the economy. In this study, the bluetongue disease status in Kurdistan province of Iran has been investigated.

2. MATERIALS AND METHODS

A total of 297 field sera were collected from Healthy sheep with no symptoms in Kurdistan part of Iran. The blood samples were collected from Jugular vein into vacationer tubes without EDTA. Sera were extracted by centrifugation at 2000g for 10 minute from the collected blood samples and stored at -20ºC until analysis.

2.1 c-ELISA

The anti-BTV antibodies were detected in the serum samples via a c-ELISA method using a commercial Kit (ID-Vet Innovative Diagnostics 34070 Montpellier – France). The basis of the test was centered on the competition between test sera and an anti-VP7 MAb for a VP7 antigen that was previously bound to the solid phase of the ELISA plate. The positivity/negativity of the test samples were determined from the level of inhibition recorded in relation to an anti-VP7 MAb control in the absence of test sera.

3. RESULTS AND DISCUSSION

The seroprevalence of BTV that occur within the parts of Iran are poorly defined and there have been few recent published studies from the region. Of the 297 sera tested, 126 (42.42%) were positive by BTV c-ELISA test. Significant difference was found between the prevalence rates observed in different classes of age Fig. 1, ($P<0.05$). With Australian serotypes, disease occurs only in sheep that are three years of age or older [9]. Seroprevalence increases with age, which is probably a reflection of increased duration of exposure [9,10]. In Iran, identification of BTV in suspected cattle and sheep based on clinical manifestations was performed. However, there are some limitations and problems. Firstly, it should be considered that clinical expression of BTV regarding strain and virus intensity, cattle race and environmental condition varies from per-acute to subclinical. Secondly, symptoms of disease in sheep can be mistaken with those of other viral diseases and even some of the non-viral diseases [11]. In 1972, BT was first reported in parts of Iran [12]. Since then, the infection has been recorded in a number of provinces in Iran Fig. 2, on the basis of the detection of group-specific antibodies against the virus. In 1974, a serological survey was conducted for BTV antibodies in the sheep, goat, cattle, camel, Water buffaloes and Pigs populations in Iran, using Immuno-gel diffusion tests. The results of this survey indicated the presence of BTV antibodies in farm animals in Iran [12]. Since the initial report in 1972, several studies of BT in farm animals have been reported on the basis of serologytest Table 1.

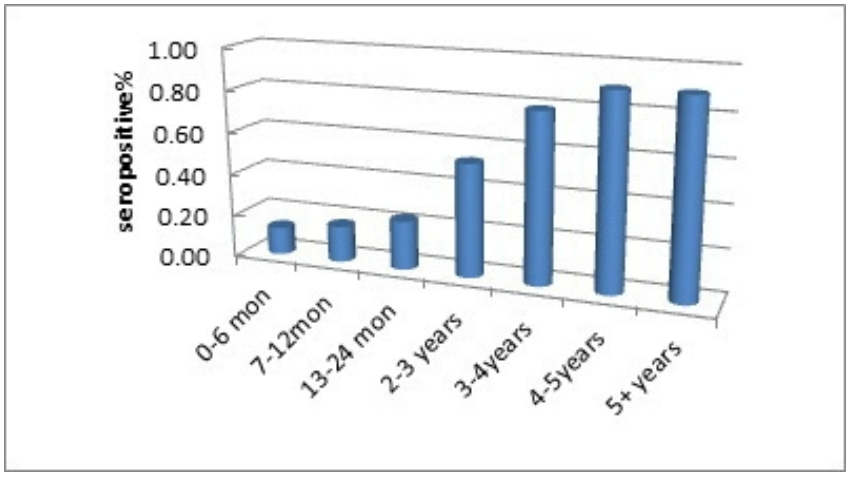

Fig. 1. Age-related seroprevalence to BTV in sheep in Kurdistan province, west of Iran, followed by an increase with age following BTV exposure and infection

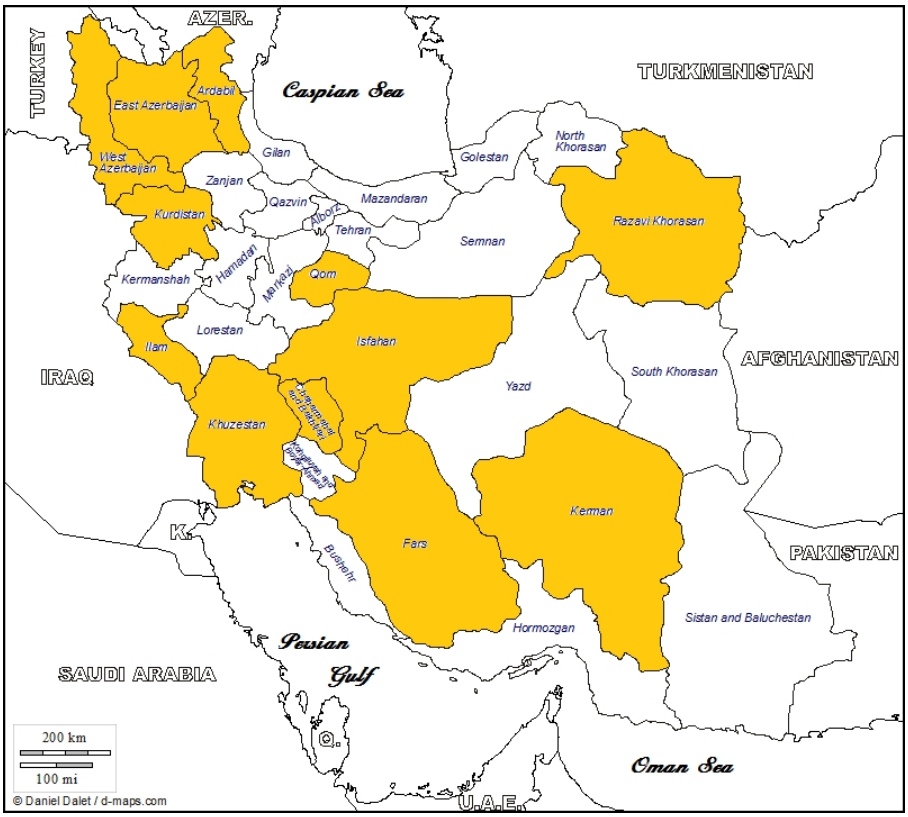

Fig. 2. Prevalence of bluetongue virus antibodies in sheep, goats, cattle, cameland pigs in various states in Iran

3.1 Sheep

There are approximately 52 million sheep in Iran [13]. Besides being used for meat and hides, milk from sheep is also consumed in Iran. 12.1 to 90% antibodies to BTV in sheep were reported [14,15]. The first evidence of BT in sheep was recorded by Hesami and Ghabousi in 1972. They reported that the disease had been suspected in sheep by some government veterinarians in parts of Iran. Their diagnosis was based on the clinical findings [12]. BTV infection has been reported from Iran's neighboring countries, e.g., Pakistan and Turkey, and it is therefore not surprising that bluetongue virus antibodies were detected in the sera of some domestic animals, reflecting the occurrence of infection among animals in Iran [12]. Although typical BT has not been reported, there are several reports of the presence of BTV antibodies in sheep in a number of states. In a serological survey conducted by Afshar and Kayvanfar in Shiraz and Tehran, 7.6% sheep was positive for BTV antibodies using Immuno-gel diffusion tests [12]. Noaman et al. reported the prevalence rate of 53.37% in sheep in Isfahan, using ELISA test [16]. In west- Azerbaijan province reported the prevalence rates were 63.1, 34.47,64.86, and 55.9% in 2007, 2010, and 2012, respectively [14,17-19]. Infection rate of 76.44 and 39.89% were reported in east- Azerbaijan in 2008 and 2012 [20,21]. In 2008, Khezri and Azimi reported that the prevalence of BTV antibodies in sheep in Kurdistan province was 51.85% [22]. Infection rate of 19.3% was reported in Kurdistan (Sanandaj) in 2011 [23]. The prevalence rate of 33.3, 43.88, 23.77, 15,

90 and 12.1% were reported in Kerman, Ilam, Ardabil, Khorasan Razavi, and Gom, respectively [9,14,24]. Infection rate of 25.35, 74.4 and 72.4% were reported in Fars province in southern Iran [14,25,29]. In Chaharmahal, Khuzestan and Isfahan, infection rats were 30.38% [11].These studies indicated that abortion in small ruminants can be remarkable as a sign in BTV infection [25-27].

Table 1. Results of BTV infection in Iran since 1972

Province	Year	Animal host	% Seroprevalence	References
Tehran, Shiraz	1974	Sheep, Goat, Cattle, Camel, pigs, water-buffalo	7.6, 13.6, 0.6, 5.9, 4.5, 0.0	12
Isfahan	2006	Sheep, Goat	53.37, 49.19	16
Kerman	2006	Camel	100	34
East Azerbaijan	2008	Sheep	76.44	21
West Azerbaijan	2009	Sheep	63.1	17
West Azerbaijan	2009	Sheep	34.7	18
Kerman	2010	Cattle	2.13	32
Chaharmahal, Khuzestan, Isfahan	2011	Sheep	30.38	11
Kurdistan (Sanandaj)	2011	Sheep	19.3	23
Fars	2012	Sheep, Goat	74.2, 72.9	29
Kurdistan	2012	Sheep	51.85	22
West Azerbaijan	2012	Sheep	55.9	19
Kerman	2012	Sheep	6.57	9
Kerman	2012	Goat	67.7	28
Isfahan	2013	Cattle	2.69	33
Kurdistan, Ilam	2013	Sheep	46.10	20
Fars	2013	Sheep, Goat	74.4, 85.3	25
Khuzestan, Qum, Ardabil, Ilam, Fars, Kurdistan	2013	Sheep	34.93	14
W. Azerbaijan, E. Azerbaijan, Khorasan Razavi	2013	Sheep, Goat	90, 87.6	15
Ardabil, W. Azerbaijan, E. Azerbaijan	2013	Sheep	33.75	20

3.2 Goats

There are approximately 26 million goats in Iran [13]. Besides being used for meat and milk, hair from goats is also consumed in Iran. The first evidence of BT in goats was recorded by Hesami and Ghabousi [12]. Although typical BT has not been reported, there are several reports of the presence of BTV antibodies in goats in a number of states. Some studies have been carried out in different regions of the country that mostly reported high prevalence of BT. In 1974, Afshar et al. reported the prevalence rate in Tehran and Shiraz were 13.6%

[12]. In Isfahan, the prevalence rate was 49.19% [16]. In Khorasan Razavi and Kerman the infection rate were 87.6 and 67.7% [15,28]. In Fars, the prevalence rates were 74.2 and 85.3% that shows high prevalence [29,25]. This reported on the basis of serology test. These results showed that the prevalence is higher in goats than sheep. However, clinical BT disease was not observed in goats. The role of goat populations in the maintenance of BTV in nature is not known [30].Belbis et al. showed that BTV-8 transplacental transmission can occur in goats that have been infected at 61 days of pregnancy, with infectious virus recovered from the Caprine fetuses [31].

3.3 Cattle, Camel and Pigs

The evidence of BTV infection in cattle in Iran was documented in 1974 [12]. According to this report, 0.6% of cattle sera were positive for BTV antibodies. In Kerman, the prevalence rate was 2.13% [32]. Noaman et al. reported the prevalence rate of 2.69% in cattle in Isfahan [33]. In 1974, the occurrence of precipitating antibodies to bluetongue virus antigens in sera of four of 67 camels (5.9%) and one of 22 pigs (4.5%) could be indicative of previous exposure of these animals to bluetongue virus or in apparent infections [12]. In 2006, serological survey was conducted by Mahdavi et al. on a camel farm in Kerman province. The presence of BTV antibodies was detected in 100% of camel sera by ELISA test [34]. However, no attempts have been made to date to isolate BTV from semen. No studies have been conducted on the semen quality of bulls and camel with BTV or BTV antibodies. No clinical BT has been observed in cattle and camel. 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 [25] but these animals may play an important role in the maintenance of the virus in nature [30]. The presence of BTV antibodies in cattle and camel has become a serious impediment to the export of the germplasm of these animals [30].

3.4 Other Animals

The presence of BTV antibodies has been reported in several wild ruminants from different countries of the world [35]. However, no systematic survey has yet been conducted to assess the status of BTV infection in Iran wildlife.

3.5 Bluetongue Virus Serotypes Reported from Iran

Bluetongue virus is a non-enveloped virus with a genome of approximately 19200 base pairs composed of ten linear segments of double-stranded RNA (dsRNA) [36]; this makes prone to frequent mutation and genetic reassortments leading to the emergence of a new serotype or antigenic variant of the same serotype. To date, 26 serotypes of BTV have been recorded world-wide, 7 of which (serotypes 3, 4, 7, 9, 16, 20 and 22) have been reported in Iran [37-39].

3.6 Vector

Although >1000 species of *Culicoides* are known worldwide, relatively few of these species have been incriminated as vectors of BTV [40]. Species of vector insects that transmit BTV differ amongst regions, and are especially poorly characterized in the portions of Asia that are devoid of *Culicoides imicola* (*C. imicola*), the traditional African-Asian vector of BTV [18]. BTV is transmitted between its ruminant hosts almost exclusively through the bites of the

females of vector species of the *Culicoides* biting midge [41,42]. The global distribution of BTV, therefore, is restricted to those regions where these vector species of *Culicoides* occur, and its transmission period is limited to the period when adult vectors are active. Depending on the species, adult vector activity generally starts some time in spring. Activity is positively correlated with temperature and reaches a maximum between 28ºC and 30ºC; activity decreases when the temperature drop and, for the traditional Afro-Asiatic vector *C. imicola*, is probably nonexistent at temperatures <10ºC [41,43]. There is a complete lack of information on the BTV vector in Iran. Therefore, an extensive survey for identification of the insect vectors of BTV is imperative in order to understand the dynamics of the BTV maintenance cycle in nature [30].

3.7 Laboratory Methods for the Isolation of Bluetongue Virus and Detection of Antibodies in Serum

It is worth mentioning that the situation of diagnosis of this virus in neighboring countries and the Middle-East (except Turkey and Israel) is not better than our country. In such countries as Saudi Arabia, Syria, Yemen, Oman and Pakistan, the presence of the virus has been documented only relying on serological tests [11,18,44]. Many methods have been employed in order to detect BTV. The most important of them are isolation of virus via embryonic egg or cell culture, serological and molecular methods. Among these laboratory diagnostic methods, molecular techniques enjoy the highest level of sensitivity and specificity for diagnosis of Orbiviruses including BTV. This method is so sensitive that even 6 molecules of the virus genome in blood can be traced [11]. Reverse-transcription polymerase chain reaction (RT-PCR) technology has permitted rapid amplification of BTV RNA in clinical samples, and RT-PCR based procedures are now available. These procedures can augment the classical virological techniques to provide information on virus serogroup, serotype and topotype [7]. Biteau-Coroller et al. and Afshar, in their studies introduced the PCR method as a 'good test' for BTV diagnosis compared with other procedures [12,45]. Azimi et al. reported that, BTV were diagnosed in some suspected animals by RT-PCR and nested PCR, by targeting S7 segment [36]. Momtaz et al. reported that, PCR technique was employed as a quick, sensitive and specific method for diagnosis of BTV from the cases suspected to disease. They reported the detection of 769 bp segment relates to BT positive samples in Nested-PCR assay [11]. Esmaeili et al. reported that 4625 aborted fetuses of sheep and goat flocks were examined for viral and bacterial agents during 2002-2004. The results showed that 15.4% BTV were identified as abortion related agents [27]. Several diagnostic tests have been developed for the diagnosis of BTV infection in domestic and wild ruminants [7], among the various serogroup-specific assays for antibody detection, the agar gel immunodiffusion (AGID) and c-ELISA are the most widely used tests [12]. In Iran, most of the serological studies have been conducted using c-ELISA.

3.8 Control

Iran is thus immediately adjacent to the unstable BT zone involving Afghanistan, Iraq, Pakistan and Turkey [46]. The economy of the area is based on agriculture and domestic ruminants come into contact when grazing on extensive semi-arid rangeland pastures [14,34,46]. When taking into consideration the seasonal movements of different animals, it is suggested that risk-based control measures be adopted [44]. Iran is located in the South-East of Europe; this makes it an important potential source of BTV strains and serotypes that may spread to adjacent countries [43]. The distribution and intensity of infection in regions of the continents is determined by the climate, geography and altitude, since these factors

affect the occurrence and activity of the *Culicoides* vectors. The presence of susceptible mammalian hosts also plays an important role [43]. Climate is a major risk factor as *Culicoides* require warmth and moisture for breeding and calm and warm humid weather for feeding [43]. In Iran there is no well-defined control strategy for BT as in other countries. Vaccination is not performed against BTV in Iran, mainly due to non-availability of the vaccine. Moreover, the exact number of serotypes circulating in Iran is still not known. With regard to vector control, no attempt for control has been made on any farm in this country. There is no restriction on the movement of animals from one region to another within the country. Thus, outbreaks may also occur due to transportation of animals. 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 sub-clinically 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 [47]. Introduction of BTV from 1 area into another can occur in 4 ways: through animal movement (domestic and wild ruminants) or animal product transport (semen, embryos); by infected vector *Culicoides spp.* carried by various living (plants, animals) or inanimate (airplanes, ships) means; through the active flight of infected vector *Culicoides spp.* (local propagation); and through passive flight of infected vector *Culicoides spp.* on the wind (responsible for long distance dissemination) [42]. Whether the virus becomes established in a new area depends upon the number and distribution of susceptible hosts, the duration and titer of the BTV viremia in the hosts, the vector capacity of the local vector population, and the ambient temperature. In essence, establishment depends upon a sufficient number of vector *Culicoides spp.* becoming infected by feeding upon local viremic hosts, surviving long enough to ensure completion of the intrinsic incubation period (4–20 days),and transmitting the virus by bite to new hosts [41,42]. The extrinsic incubation period is the interval between when a vector is infected and when it first becomes capable of transmitting the BTV to a new host [41,42]. It also reveals that many questions remain regarding the physiopathological mechanisms of the BTV induced thrombo-haemorrhagic disease, the species/ breeds/ individual genetic bases of sensitivity to BT disease, the molecular basis of BTV virulence, the insect and mammalian reservoirs, and the immune effectors involved in cross protective immunity. Although classical vaccination theoretically can control BT, major economic constraints may prevent its effectiveness, due to the huge vaccine supplies necessary to insure a >80% coverage in ruminants, the costs inherent in the use of inactivated vaccines, the lack of cross serotype protection in face of possible new serotype invasions and the different policies applied in neighboring countries [47]. Consequently, a well-defined control strategy for preventing and controlling BT may be based not only on vaccination plans and vector eradication but also restriction on the movement of animals from one region to another within the country.

4. CONCLUSION

This study demonstrated that increase in the susceptible population; along with favorable the most parsimonious model showed a significant non-linear increase in seroprevalence with age, appears to have led to the establishment of BT in Kurdistan Province. Further research on the isolation and identification of BTV in *Culicoidesspp*. are encouraged.

CONSENT

No applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the ethics committee of Razi Vaccine and Serum Research Institute, Alborz, Iran.

ACKNOWLEDGEMENTS

We are grateful to Razi Vaccine and Serum Research Instituted.

COMPETING INTERESTS

The authors declare that no competing interests exist.

REFERENCES

1. Monaco F, Cammà C, Serini S, Savini G. Differentiation between field and vaccine strain of bluetongue virus serotype 16. Veterinary Microbiology. 2006;116(1-3):45-52.
2. Verwoerd DW, Erasmus BJ. Bluetongue. In Infectious Diseases of Livestock, 2th ed. Cape Town, South Africa: Oxford University Press; 2004.
3. Parsonson IM. Pathology and pathogenesis of bluetongue infections. Current Topicsin Microbiology and Immunology. 1990;162:119-141.
4. Melville LF, Hunt NT, Davis SS, Weir RP. Bluetongue virus dose not persist in naturally infected cattle. Veterinaria Italiana. 2004;40:502-507.
5. Hofmann MA, Renzullo S, Mader M, Chaignat V, Worwa G, Thuer B. Genetic characterization of Toggenburg Orbivirus, a new bluetongue virus, from goats, Switzerland. Emerging Infectious Disease Journal. 2008;14(12):1855-1861.
6. Maan S, Maan NS, Nomikou K, Batten C, Antony F, Belaganahalli MN, et al. Novel bluetongue virus serotype from Kuwait. Emerging Infectious Disease Journal. 2011;17(5):886-889.
7. Office International des Epizooties (OIE). Infection with bluetongue viruses. In: Meeting of the OIE terrestrial animal health standards commission. Paris, OIE. 2011;193-209.
8. Office International des Epizooties (OIE). OIE-Listed diseases, infections and infestations in force in 2013.
   Available: http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2013/
9. Mozaffari AA, Khalili M. The first survey for antibody against Bluetongue virus in sheep flocks in Southeast of Iran. Asian Pacific Journalof Tropical Biomedicine. 2012;2(3):1808-1810.
10. Radostitis M, Gay CC, Hinchcliff KW, Constable PD. Veterinary Medicine. 10th ed. Saunders; 2007.
11. Momtaz H, Nejat S, Souod N, Momeni M, Safari S. Comparisons of competitive enzyme-linked immunosorbent assay and one step RT-PCR tests for the detection of Bluetongue virus in south west of Iran. African Journal of Biotechnology. 2011;10(36):6857-6862.
12. Afshar A, Kayvanfar H. Occurrence of precipitating antibodies to bluetongue virus in sera of farm animals in Iran. Veterinary Record. 1974;94(11):233-235.
13. Agriculture Mo. A Statistics of Agriculture. 2011;2.
    Available at:http://dpe.agri-jahad.ir/portal/File/ShowFile.aspx?ID=75ea3522-343d-4719-a8c2-d81cf11108e9

14. Khezri M, Azimi SM. Epidemiological investigation of bluetongue virus antibodies in sheep in Iran. Veterinary World. 2013;6(3):122-125.
15. Najarnezhad Mashadi V, Rajai M, Azghadi NM, Rashtibaf M, Alami J. A seroepidemiological study of Bluetongue virus in sheep and goats in Khorasan Razavi. In: The 1st international congress of large animal practitioners (ICLAP). vol. 1, Tehran-Iran. 2011;46.
Available:http://parasites-world.com/1st-international-congress-of-large-animal-practitioners iclap-2011/
16. Noaman V, Kargar Moakhhar R, Shah Moradi AH, Hydari MR, Tabatabaei J. A Seroepidemiological survey for blue-tongue virus antibody in sheep and goats of Isfahan province, Iran; 2006.
Available: www.agris.fao.org/agris-search/search/display.do?f=2012/DJ/...xml
17. Bokaie S, Rouhani Kargar M, Mousavi M, Sharifi L, Ramin AG, Aras Khani A. Serological survey on bluetongue disease in suspicious sheep flocks in West Azerbaijan province, Iran. Iranian Veterinary Journal. 2007;3(3):81-84.
18. Jafari-Shoorijeh S, Ramin AG, Maclachlan NJ, Osburn BI, Tamadon A, Behzadi MA, et al. High seroprevalence of bluetongue virus infection in sheep flocks in West Azerbaijan, Iran. Comparative Immunology, Microbiology and Infectious Diseases. 2010;33(3):243-247.
19. Sadri R. Seasonal effects on the prevalence of bluetongue in small ruminants in west Azerbaijan, Iran. Iranian Journalof Veterinary Medicine. 2012;6(14):19-22.
20. Khezri M, Azimi SM. Seroprevalence of bluetongue disease in sheep in west and northwest provinces of Iran. Veterinary Research Forum. 2013;4(3):195-198.
21. Hasanpour A, Mosakhani F, Mirzaii H, Mostofi S. Seroprevalence of Bluetongue Virus Infection in Sheep in East-Azerbaijan Province in Iran. Research Journal of Biological Sciences. 2008;3(11):1265-1270.
22. Khezri M, Azimi SM. Investigation of bluetongue virus in Kurdish sheep in Kurdistan province of Iran. African Journal of Microbiology Research. 2012;6(35):6496-6501.
23. Khanbabaie H, Fakur S, Khezri M, Mohammadian B, Rokhzad B. Serological survey of Bluetongue disease in sheep of Sanandaj city by ELISA. Journal of Veterinary Medicine. 2011;5(2):11-18.
24. Najarnezhad V, Rajae M, Batavani RA. Seroepidemiology of bluetongue disease in small ruminants of north-east of Iran. Asian Pacific Journal of Tropical Biomedicine. 2013;3(6):492-495.
25. Oryan A, Amrabadi O, Mohagheghzadeh M. Seroprevalence of bluetongue in sheep and goats in southern Iran with and overview of four decades of its epidemiological status in Iran. Comparative Clinical Pathology. 2013;22(97):1991-2013.
26. Mostaghni K. The incidence of some pathogenic organisms associated with abortion in ewes in Iran. Indian Veterinary Journal. 1980;57:624-626.
27. Esmaeili H, Ebrahimzadeh H, Khalaj M, Rasoli beirami N, Gholami H, Khaji L, et al. Infectious agents of abortion in sheep and goat flocks of Iran. In: The 1st international congress of large animal practitioners (ICLAP). vol. 1. Tehran-Iran. 2011;192.
Available: http://parasites-world.com/1st-international-congress-of-large-animal practitioners-iclap-2011/
28. Mozaffari AA, Khalili M, Sabahi S. High seroprevalence of bluetongue virus (BTV) antibodies in Goats in Southeast Iran. Asian Pacific Journal of Tropical Biomedicine. 2012;1-6.

29. Mohammadi A, Tanzifi P, Nemati Y. Seroepidemiology of bluetongue disease and risk factors in small ruminants of Shiraz suburb, Fars province, Iran. Tropical Biomedicine. 2012;29(4):632-637.
30. Prasad G, Jain NC, Gupta Y. Bluetongue virus infection in India: a review. Scientific and Technical Review of the Office International des Epizooties. 1992;11(3):699-711.
31. Belbis G, Bréard B, Cordonnier N, Moulin V, Desprat A, Sailleau C, et al. Evidence of transplacental transmission of bluetongue virus serotype 8 in goats.Veterinary Microbiology. 2013;166(3–4):394-404.
32. Mozaffari AA, Khalili M, Yahyazadeh F. A serological investigation of bluetongue virus in cattle of south-east Iran. Veterinaria Italiana. 2012;48(1):41-44.
33. Noaman V, Shirvani E, Hosseini SM, Shahmoradi AH, Heidari MR, Raiszadeh H, Kamalzadeh M, Bahreyari M. Serological surveillance of bluetongue virus in cattle in central Iran. VeterinariaItaliana. 2013;49(2):141-144.
34. Mahdavi S, Khedmati K, Pishraft Sabet L. Serologic evidence of bluetongue infection in one-humped camels (Camelusdromedarius) in Kerman Province, Iran. Iranian Journal of Veterinary Research. 2006;7(3):85-87.
35. Falconi C, López-Olvera JR, Gortázar C. BTV infection in wild ruminants, with emphasis on red deer: a review. Veterinary Microbiology. 2011;151(3-4):209-219.
36. Mertens PP, Sangar DV. Analysis of the terminal sequences of the genome segments of four Orbiviruses. Virology. 1985;140:55-67.
37. Moakhar RK, Taylor WP, Ghaboussi B, Hessami M. Serological survey of sheep in Iran for type specific antibody 10 bluetongue virus. Archivesof Razi Institute. 1988;38:92-99.
38. Azimi SM, Keyvanfar H, Pourbakhsh SA, Razmaraii N. S7 gene Characterization of bluetongue viruses in Iran. Archivesof Razi Institute. 2008;63(1):15-21.
39. Khezri M, Azimi SM. Seroprevalence and S7 gene characterization of bluetongue virus in west of Iran. Veterinary World. 2012;5(9):549-555.
40. Meiswinkel R, Gomulski LM, Delecolle JC, Goffredo M, Gasperi G. The taxonomy of *Culicoides* vector complexes unfinished business. Veterinaria Italiana. 2004;40:151-159.
41. Mellor PS, Boorman J, Baylis M. Culicoides biting midges: their role as arbovirus vectors. Annual of Review Entomology. 2000;45:37-40.
42. Saegerman C, Berkvens D, Mellor PS. Bluetongue epidemiology in the European Union. Emerging of Infectious Disease. 2008;14:539-544.
43. Purse BV, Mellor PS, Rogers DJ, Samuel AR, Mertens PP, Baylis M. Climate change and the recent emergence of bluetongue in Europe. Natures Review Microbiology. 2005;3(2):171-181.
44. Akhtar S, Djallem N, Shad G, Thiemo O. Bluetongue virus seropositivity in sheep flocks in North West Frontier Province, Pakistan. Preventive Veterinary Medicine. 1997;29(4):293-298.
45. Biteau-Coroller F, Gerbier G, Stark KDC, Grillet C. Performance evaluation of competitive ELISA test used for bluetongue antibody detection in France, a recent infected area. Veterinary Microbiology. 2006;118:57-66.
46. Mellor PS, Carpenter S, Harrup L, Baylis M, Mertens PP. Bluetongue in Europe and the Mediterranean Basin: history of occurrence prior to 2006. Preventive Veterinary Medicine. 2008;87:4-8.

47. Nikolakaki SV, Nomikou K, Koumbati M, Mangana O, Papanastassopoulou M, Mertens PP, et al. Molecular analysis of the NS3/NS3A gene of Bluetongue virus isolates from the 1979 and 1998-2001 epizootics in Greece and their segregation into two distinct groups. Virus Research. 2005;114:6-14.

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