

# A serological investigation of bluetongue virus in cattle of south-east Iran

Ali Asghar Mozaffari<sup>(1)</sup>, Mohammad Khalili<sup>(2)</sup> & Farhang Yahyazadeh<sup>(3)</sup>

## Summary

The aim of this study was to describe the seroprevalence rate of bluetongue virus (BTV) in cattle herds in south-east Iran. A total of 188 serum samples were collected from 20 cattle herds (10 animals in each herd) that were randomly selected between 2009 and 2010. A total of 12 samples were eliminated because of inadequacy. Antibodies to BTV in sera were detected using a commercial competitive enzyme-linked immunosorbent assay (c-ELISA). The seroprevalence rate in cattle was 2.13%. All sampled animals were female and age did not affect the prevalence of infection.

## Keywords

Bluetongue, Bluetongue virus, Cattle, Competitive ELISA, Enzyme-linked immunosorbent assay, Iran, Virus.

## Virus della bluetongue: indagine sierologica nel bestiame del territorio a sud-est dell'Iran

### Riassunto

*Obiettivo dello studio è stata la descrizione del tasso di sieroprevalenza del virus della bluetongue (BTV) nel bestiame del territorio a sud-est dell'Iran. Sono stati raccolti 188 campioni di siero da animali di sesso femminile di 20 mandrie (10 capi per mandria), selezionate in modalità random tra il 2009 e il 2010. Dodici campioni sono stati eliminati poiché inadeguati. Gli anticorpi relativi al BTV*

*sono stati evidenziati mediante test con immuno-assorbente legato all'enzima competitivo (c-ELISA) disponibile in commercio. Il tasso di sieroprevalenza nel bestiame è risultato pari al 2,13%. L'età non ha influito sulla prevalenza dell'infezione.*

## Parole chiave

Bestiame, Bluetongue, BTV, c-ELISA, Iran, Test con immuno-assorbente, Virus della bluetongue.

## Introduction

Bluetongue (BT) was first reported more than 125 years ago, when European breeds of sheep were introduced into southern Africa. BT viruses (BTV) have been identified in many tropical and temperate areas of the world. BT, the disease, is a phenomenon of ruminants in the temperate zones. There is little clinical disease in the tropical and subtropical areas of the world (21). Bluetongue is a notifiable disease of the World Organisation for Animal Health (*Office of International Epizootics*: OIE) (25). It is listed with those diseases that can spread rapidly and that have a considerable impact on the health of livestock (9, 25). BTV is an arthropod-borne orbivirus that causes infection in wild and domestic ruminants (27). At least 24 serotypes of BTV have been identified worldwide (6) that can cause BT as either an acute, subacute, mild or inapparent disease. BTV can infect several species of domestic and wild ruminants. Sheep are the most susceptible species (13). In cattle and goats, infection occurs mostly asymptotically (14). Cattle might act as a reservoir of infection

(1) Department of Clinical Studies, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran  
aliasghar\_mozaffari@uk.ac.ir, mozafari313@yahoo.com

(2) Department of Pathobiology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

(3) Graduated, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

although some authors have indicated that BTV does not persist in naturally infected cattle (12, 24). The disease may cause clinical signs of fever, oral lesions, excessive salivation, lameness, abortion, infertility and congenital deformities (1). However, loss of body weight and condition, drop in milk production and poor subsequent reproductive performance and indirect losses associated with the prohibition of livestock trade from infected countries were thought to have a greater economic effect than occasional overt disease (2).

Worldwide, BTV has been estimated to cause direct (disease) and indirect (trade, vaccines, etc.) losses of over US\$3 billion per year (11). Various techniques have been used to detect antibodies against BTV. These include agar gel immunodiffusion (AGID), haemagglutination-inhibition, complement fixation, competitive-ELISA (c-ELISA) and serum neutralisation, which is the only serotype-specific assay. The c-ELISA is the serological method recommended as a prescribed test for international trade in the OIE *Manual of standards for diagnostic tests and vaccines* (25). When the BT c-ELISA was used, the cross-reactions disappeared (18). Despite the significant advances that have been made in molecular techniques, the traditional approach using biology-based test procedures is still the mainstay for the laboratory confirmation of clinical diagnosis (8). The objective of our study was to describe the seroprevalence rates of BTV in cattle in south-east Iran.

## Materials and methods

A total of 188 serum samples were collected from 20 randomly selected herds of cattle (10 animals per herd) between 2009 and 2010 in the north, north-west, central and southern parts of south-east Iran. Twelve samples (belonging to 12 herds) were eliminated because of inadequacy. The sampled animals were 2-5 year-old females. In each herd, 3 animals aged 2-3 years, 3 aged 2-4 years and 4 aged 4-5 years were randomly sampled. Blood was collected into sterile tubes by jugular vein puncture. Blood samples were

centrifuged and sera were gathered and stored at  $-20^{\circ}\text{C}$ . Antibodies to BTV in sera were detected by using a commercial c-ELISA (Institut Pourquier, Montpellier) in accordance with the instructions of the manufacturer. One-way analysis of variance (ANOVA) (SPSS v12; SPSS Inc, Chicago, Illinois) was used to compare seroprevalence rate data between different age groups. The 95% confidence interval was calculated for each prevalence rate.

## Results

BTV antibodies were detected in 4 cattle which belonged to four herds in the southern area of the south-east Iran. The overall seroprevalence rate was 2.13% for cattle (95% CI = 0.86-5.33%) whereas the herd prevalence was 20% (95% CI = 8.22-41.91%). No significant difference was found between the prevalence rates observed in the different classes of age.

## Discussion

In our study, the seroprevalence rate was 2.13% for cattle. The first evidence of BT disease in 10 pregnant camels from south-east Iran was reported by Mahdavi *et al.* in 2006 (10). A high seroprevalence (34.7%) of BTV infection has been reported in sheep flocks in West Azerbaijan, Iran. In this survey, 172 of 184 flocks included BTV seropositive sheep (93.5%) (19). BTV seropositivity rates in sheep were recorded at 29.5% in south-eastern Turkey (7). BTV seropositive reactions were obtained in 184 (48.4%) out of 380 tested sera and in 89.5% (34/38) of the sheep flocks in the North West Frontier Province of Pakistan. In 34 seropositive flocks, the prevalence ranged from 12.5% to 100% (median = 47) (1).

BTV is currently recognised as infecting domestic ruminants in Africa, Asia, North America and Australia and several islands. As a general rule, one can now consider that BTV infects livestock populations in all countries lying in the tropics and sub-tropics. The current range for BT disease lies between the latitude of  $53^{\circ}\text{N}$  and  $34^{\circ}\text{S}$  (26). Iran is immediately adjacent to the BT zone where the

situation (existence of disease) is unstable (Afghanistan, Iraq, Pakistan and Turkey) (11). South-east Iran lies within the above-mentioned range (10). Thus, the occurrence of BTV infection in south-east Iran is expected. The agricultural economy in that area is based on pasture in extensive semi-arid rangeland; therefore, domestic ruminants come into contact when grazing (10, 11). Considering the seasonal movements of different live animals, it is suggested that a risk-based approach be adopted (1).

The distribution and intensity of infection in regions of the continents is determined by the climate, geography and altitude, as they might affect the occurrence and activity of the *Culicoides* vectors and by the presence of susceptible mammalian hosts (4, 5, 16). Climate is a major risk factor as *Culicoides* require warmth and moisture for breeding and calm, warm humid weather for feeding (16). A cold winter or a dry summer can markedly reduce vector numbers and risk for diseases. Moisture may be in the form of rivers and streams or irrigation but rainfall is the predominant influence and rainfall in the preceding months is a major determinant of infection. Optimal temperatures are also essential and, in endemic areas, temperatures for survival of the adults and larvae should constantly be above a mean of 12.5°C for the cooler months and in the range of 18°C to 30°C in the summer and autumn. These values guarantee optimum recruitment of adults and optimal activity (15, 20, 22, 23).

The climate of south-east Iran varies according to region. The north, north-west and central areas experience a dry and moderate climate, whereas the south and south-east has warm weather and is relatively humid. The temperature of south-east Iran is variable with maximum and minimum temperatures of 39.6°C and -9°C, respectively. This means that the climate conditions of the south-east Iran

might be not suitable for the survival of adults and larvae of *Culicoides* vectors. The absence, in the present study, of seropositive animals in the northern areas of south-east Iran may be attributed to the dry climate and to high variations in temperatures which are not suitable for the survival of adults and larvae of *Culicoides* vectors. The presence of seropositive animals, in the present study, only in the southern areas of south-east Iran may be attributed to the warm and relatively humid climate in this area which is suitable for the survival of adults and larvae of *Culicoides* vectors.

## Conclusions

In our study, all animals sampled were over two years of age and were female. There are differences in age susceptibility to clinical disease which inexplicably vary with different outbreaks. With Australian serotypes, disease occurs only in sheep that are three years of age or older (3). Seroprevalence increases with age, which is probably a reflection of increased duration of exposure (17). Regarding the influence of sex in the prevalence of infection, it has been observed that bulls have a greater risk for infection than females or castrated males (17). The lack of significant differences between classes of age could depend on the low number of positive animals found in this study.

As a vaccination programme for BT is not implemented in Iran, a seropositive result indicates BT infection in domestic populations (11). Further research on the isolation and identification of BT virus in cattle are encouraged.

## References

1. Akhtar S., Djallem N., Shad G. & Thieme O. 1997. Bluetongue virus seropositivity in sheep flocks in North West Frontier Province, Pakistan. *Prev Vet Med*, **29**, 293-298.

2. Aradaib I.E., Mohamed M.E., Abdalla T.M., Sarr J., Abdalla M.A., Yousof M.A., Hassan Y.A. & Karrar A.R. 2005. Serogrouping of United States and some African serotypes of bluetongue virus using RT-PCR. *Vet Microbiol*, **111**, 145-150.
3. Bishop A.L., Kirkland P.D., McKenzie H.J. & Barchia I.M. 1996. The dispersal of *Culicoides brevitarsis* in eastern New South Wales and associations with the occurrences of arbovirus infections in cattle. *Aust Vet J*, **73**, 174-178.
4. Braverman Y. & Chechik F. 1996. Air streams and the introduction of animal diseases borne on *Culicoides* (Diptera, Ceratopogonidae) into Israel. *Rev Sci Tech*, **15**, 1037-1052.
5. Gibbs E.P. & Greiner E.C. 1994. The epidemiology of bluetongue. *Comp Immunol Microbiol Infect Dis*, **17**, 207-220.
6. Gorman B.M. & Roy P. 1990. The bluetongue viruses. Springer, Berlin, 162 pp.
7. Gür S. 2008. A serologic investigation of blue tongue virus (BTV) in cattle, sheep and gazella subgutturosa subgutturosa in southeastern Turkey. *Trop Anim Health Prod*, **40**, 217-221.
8. Hamblin C. 2004. Bluetongue virus antigen and antibody detection, and the application of laboratory diagnostic techniques. *Vet Ital*, **40**, 538-545.
9. Lundervold M., Milner-Gulland E.J., O'Callaghan C.J. & Hamblin C. 2003. First evidence of bluetongue virus in Kazakhstan. *Vet Microbiol*, **92**, 281-287.
10. Mahdavi S., Khedmati K. & Pishraft Sabet L. 2006. Serologic evidence of bluetongue infection in one-humped camels (*Camelus dromedarius*) in Kerman Province, Iran. *Iranian J Vet Res*, **7**, 85-87.
11. Mellor P.S. & Wittmann E.J. 2002. Bluetongue virus in the Mediterranean Basin 1998-2001. *Vet J*, **164**, 20-37.
12. Melville L.F., Hunt N.T., Davis S.S. & Weir R.P. 2004. Bluetongue virus does not persist in naturally infected cattle. *Vet Ital*, **40**, 502-507.
13. Osburn B.I. 1994. Bluetongue virus. *Vet Clin North Am Food Anim Pract*, **10**, 547-560.
14. Parsonson I.M. 1990. Pathology and pathogenesis of bluetongue infections. *Curr Top Microbiol Immunol*, **162**, 119-141.
15. Paweska J.T., Venter G.J. & Mellor P.S. 2002. Vector competence of South African *Culicoides* species for bluetongue virus serotype 1 (BTV 1) with special reference to the effect of temperature on the rate of virus replication in *C. imicola* and *C. bolitinos*. *Med Vet Entomol*, **16**, 10-21.
16. Purse B.V., Mellor P.S., Rogers D.J., Samuel A.R., Mertens P.P. & Baylis M. 2005. Climate change and the recent emergence of bluetongue in Europe. *Nat Rev Microbiol*, **3**, 171-181.
17. Radostits O.M., Gay C.C., Blood D.C. & Hinchcliff K.W. 2007. Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. WB Saunders Company, London, 1 303 pp.
18. Shimizu S., Toyota I., Arishima T. & Goto Y. 2004. Frequency of serological cross-reactions between Ibaraki and bluetongue viruses using the agar gel immunodiffusion test. *Vet Ital*, **40**, 583-586.
19. Shoorijeh S.J., Ramin A.G., Maclachlan N.J., Osburn B.I., Tamadon A., Behzadi M.A., Mahdavi M., Araskhani A., Samani D. & Rezajou N. 2010. High seroprevalence of bluetongue virus infection in sheep flocks in West Azerbaijan, Iran. *Comp Immunol Microbiol Infect Dis*, **33**, 243-247.
20. Tatem A.J., Baylis M., Mellor P.S., Purse B.V., Capela R., Pena I. & Rogers D.J. 2003. Prediction of bluetongue vector distribution in Europe and North Africa using satellite imagery. *Vet Microbiol*, **97**, 13-29.
21. Walton T.E. 2004. The history of bluetongue and a current global overview. *Vet Ital*, **40**, 31-38.
22. Ward M.P. 1994. Climatic factors associated with the prevalence of bluetongue virus infection of cattle herds in Queensland, Australia. *Vet Rec*, **134**, 407-410.
23. Ward M.P. & Thurmond M.C. 1995. Climatic factors associated with risk of seroconversion of cattle to bluetongue viruses in Queensland. *Prev Vet Med*, **24**, 129-136.
24. White D.M. & Mecham J.O. 2004. Lack of detectable bluetongue virus in skin of seropositive cattle: implications for vertebrate overwintering of bluetongue virus. *Vet Ital*, **40**, 513-519.
25. World Organisation for Animal Health (Office of International Epizootics: OIE) 2008. Manual of standards for diagnostic tests and vaccines, Sixth Ed. OIE, Paris, 1 343 pp.
26. World Organisation for Animal Health (Office of International Epizootics: OIE) 2010. Bluetongue, Chapter 8.3. Terrestrial Animal Health Code, Nineteenth Edition, OIE, Paris, 448-463.
27. Zhou E.M., Ridd D., Riva J., Fernando L. & Clavijo A. 2001. Development and evaluation of an IgM-capture ELISA for detection of recent infection with bluetongue viruses in cattle. *J Virol Methods*, **91**, 175-182.