

DETECTION OF ANTIBODIES AGAINST BLUE TONGUE VIRUS IN YAKS (*BOS GRUNNIENS*) IN ISSYK KUL, FIRST REPORT

O. Avci, Y. Orhan*, B. Oya, K. Mehmet**, Y. Sibel, and S. Atilla

Department of Virology, Faculty of Veterinary Medicine, University of Selcuk, 42003, Konya, Turkey

*Veterinary Medicine Faculty, Kyrgyzstan-Turkey Manas University, Bishkek, Kyrgyzstan

**Department of Virology, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, Burdur, Turkey

Corresponding author E-mail: oavci@selcuk.edu.tr

ABSTRACT

Bluetongue virus (BTV) caused infection is commonly determined in ruminants including sheep and cattle. However, there is a little information about the seroprevalence of BTV in yaks. The aim of this study was to describe the seroprevalence rate BTV in unvaccinated yaks in Issyk Kul, Kyrgyzstan. A total of 168 serum samples were collected from yaks between September to November 2012. Antibodies to BTV in sera were detected using a commercially available competitive enzyme-linked immunosorbent assay. 2.38% seroprevalence (n: 4) was determined in the samples as positive for BTV antibodies. This study indicates that antibodies against BTV are uncommon among the yaks in Issyk Kul. This is the first time that the seroprevalence of antibodies against BTV have been studied in yaks in Kyrgyzstan. In conclusion; despite the low results large scale research is necessary for the prevention of BTV infection.

Key words: BTV, ELISA, Yak, Antibody.

INTRODUCTION

Bluetongue virus (BTV) caused infection is a commonly defined among cattle and wild ruminants all over the world and characterized with depression, diarrhea and temporary leucopenia (Howert *et al.*, 1988; Erasmus, 1990). Bluetongue virus is an *Orbivirus* in the *Reoviridae* family (Mertens *et al.*, 2007; Attoui *et al.*, 2009) and includes 26 antigenically distinct serotypes (Chaignat *et al.*, 2009; Maan *et al.*, 2011). BTV is an icosahedral virus (80 nm in diameter) with a 10-segmented, dsRNA genome (Mellor *et al.*, 2008). The outer capsid is composed of two proteins, VP2 and VP5, which responsible for attachment to cell receptor, hemagglutination, and producing serotype-specific neutralizing antibodies (Hassan *et al.*, 2001; Zhou *et al.*, 2001). The *orbiviruses* may cause many economically important viral diseases in sheep, cattle and other animal species (Mellor *et al.*, 2008). Bluetongue (BT), an arthropod-borne disease, can also occur by biting midge *Culicoides* spp., infection can also replicate (Mellor, 1990). Sometimes either an oral route or vertically transmission can be observed in ruminants (Maclachlan and Guthrie, 2010). Clinical signs of BTV infection are abortion/death of lamb, torticollis, arthrogriposis and hydrancephaly, deterioration of wool, occasional cyanosis of the tongue, vascular thrombosis, reduction in milk production, ischemic necrosis, depression, anorexia, apathy, tachypnea, erosions of the mucosa of the buccal cavity, hemorrhage and vascular leakage, inflammation of the coronary band (Maclachlan *et al.*, 2008). Infection may be occurred more severe in sheep populations

(Goltz, 1978; Mahrt and Osburn, 1986; Mellor *et al.*, 2008). Goats and cattle can be considered as reservoir hosts (Maclachlan *et al.*, 2008).

Different serological assays such as Enzyme Linked Immunosorbent Assay (ELISA) (Maclachlan, 2004), serum neutralization tests (SNT) (Batten *et al.*, 2012), and agar gel immunodiffusion (AGID) can be used for diagnosis of specific antibodies against BTV. Susceptible samples need to be passage through embryonated chicken eggs and/or cell culture for isolation of BTV antigen (Anthony *et al.*, 2007; Franchi *et al.*, 2008). Real time PCR can be used for detection of BTV viral genome in *Culicoides* spp. (Deken *et al.*, 2008). Although BTV is not detected in the tissue samples, BTV RNA could be detected in blood samples by real time RT-PCR (Dijkstra *et al.*, 2008).

Commonly occurrence of BTV in ruminants such as sheep and cattle was taken into consideration, probability of BTV existence in yaks as a ruminant was hypothesized.

The aim of this study was to describe the seroprevalence rate of BTV in unvaccinated yaks in Issyk Kul, Kyrgyzstan.

MATERIALS AND METHODS

Animals and sampling: A total of 168 blood serum samples were collected from unvaccinated yaks that were randomly selected between September to November 2012 in Issyk Kul, Kyrgyzstan (Fig 1). All the operations were humane according to the animal welfare. Blood samples (5 mL) were taken from the jugular vein with sterile

vacuum tubes with kaolin. Collected blood samples packed in ice were brought to the laboratory, and centrifuged at $3000 \times g$ for 10 min for obtaining serums.

Approximately 1 mL of serum was collected into sterile microfuge tubes and stored at -20°C until analysis.



Fig 1. Map of Kyrgyzstan (study area) showing the location of provinces where yaks were sampled for antibodies against BTV.

Competitive ELISA: Antibodies to BTV in sera were detected using a commercially available c-ELISA, (VMRD, USA). The test was performed as per the manufacturer's instructions. The plates were then read spectrophotometrically with a 620 nm filter on an automatic ELISA reader (Rayto RT-2100C, China). The percent inhibition (%) values for the positive controls as well as samples were calculated.

RESULTS AND DISCUSSION

Seroprevalence of BTV is shown in Table 1. Positive antibodies for BTV were determined as 2.38% (n: 4), while 97.62% (n: 164) negative antibodies were determined.

Table 1. Seroprevalence of BTV in 168 yaks

Biometric Data	Total	Areas
Examined	168	Issyk Kul
Positive	4	Issyk Kul
Prevalence (%)	2.38	4

Bluetongue is a viral infection of both domestic and wild ruminants, transmitted by *Culicoides* spp., characterized by abortion, congestion, cyanosis of the tongue, edema and hemorrhage etc (Maclachlan *et al.*, 2008). BTV infection is mostly seen in some tropical/subtropical regions. Infection may cause severe economic losses in stock farming (Mellor *et al.*, 2008).

In this study, 2.38% seropositivity for BTV was detected by cELISA in unvaccinated yaks in Issyk Kul (Table 1). Although, cELISA could not differentiate the antibodies whether, due to infection or vaccination with live attenuated vaccine, this result showed that true

prevalence of BTV in this region since unvaccinated yaks were sampled. As a vaccination programme for BTV infection is not implemented in Kyrgyzstan, a seropositive result indicates BTV infection in yak populations. In addition, natural environment of the yak and other species are usually exposed to active *Culicoides* spp. To date, BTV antibodies have been found in many wild ruminants (Barnard, 1997; Mauroy *et al.*, 2008), and our results extend the yaks were susceptible to BTV. In a case report, BTV antigen positive by RTP-PCR was reported in a captive yak (Mauroy *et al.*, 2008). Antelopes can play a role as asymptomatic reservoir hosts in the epidemiology of BTV (Verwoerd and Erasmus, 2004). Monitoring of ranch raising domestic ruminants which include clinical inspection with serological and virological laboratory tests, and a monitoring of *Culicoides* spp. are necessary (Sperlova and Zendulkova, 2011). There is a little information about the seroprevalence of BTV in yaks in the world.

Obtained low seropositive rate may be derived from randomly sampling method and/or the sampling season. This study was applied between September to November 2012; however *Culicoides* species may not be active in the sampling time, as well (Schwartz-Cornil *et al.*, 2008). In addition, *Culicoides* spp. as vectors of BTV might not be collected and identified in this study; hence we could not obtain any knowledge about the *Culicoides* spp. In the current study, serotypes of BTV could not be determined. However, BTV have different lineages and divided into subtypes based on sequence of genome and phylogenetic analysis (Maan *et al.*, 2011). BTV-25 and BTV-26, new serotypes of BTV, have been isolated from animals with no symptom of BT (Batten *et al.*, 2013). In a short period of time, European researchers should consider vaccination to prevent the spread of the BTV infection in member countries (Saegerman *et al.*, 2007).

All premises with domestic and wild ruminants need to be involved in BTV and other viral infections control and prophylaxis (Mauroy *et al.*, 2008).

Diagnosis of BTV infection is defined with clinical signs, post-mortem findings and epidemiological assessment. However, BTV must be confirmed by laboratory diagnostic tests (Afshar *et al.*, 1989) such as AGID, cELISA and SNT (Hamblin, 2004). A competitive ELISA test targeted to the VP7 protein can be used for the detection of serogroup-specific antibodies against BTV. AGID, complement-fixation test (Afshar *et al.*, 1989), and haemagglutination-inhibition test (Boulanger and Frand, 1975) can be preferred for identified of serogroup-specific antibodies. There are some commercial ELISA kits developed recently by which early antibodies against BTV in serum, plasma or bulk milk samples can be detected (Mars *et al.*, 2010). cELISA has been used measurement of antibody in blood sera in BTV because of which can detect neutralizing antibodies against all serotypes of BTV. It has been used extensively for the determination of serum antibody after 6th post-infection day (Koumbati *et al.*, 1999). Thus, all neutralizing antibodies against to BTV were detected by cELISA test. In this study, a total of 168 yaks serum were examined by cELISA for BTV antibodies. The results showed that 4 (2.38%) of the samples were positive for BTV antibodies (Table 1).

In conclusion, antibodies against BTV are uncommon among the yaks in Issyk Kul, but yaks have a potential risk to other species. To our best knowledge, this is the first seroprevalence survey of yaks infected by BTV in Issyk Kul. Despite the low results it is necessary to carry out measures to prevent and control BTV infection in yaks. These preliminary observations should be followed by a further large-scale survey to establish the extent of BTV infection in Kyrgyzstan.

Competing interests: The authors declare that they have no competing interests.

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