Antibodies to Bluetongue, Akabane and Schmallenberg viruses in native dromedary camels in Turkey

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ABSTRACT
Sera from 86 Turkish native camels from seven provinces in Turkey were collected and tested for specific antibodies to Bluetongue virus (BTV), Akabane virus (AKAV) and Schmallenberg virus (SBV) using ELISA. The BTV, AKAV and SBV antibodies were found in 53.5%, 51.2% and 15.1%, respectively. Furthermore, the seropositivity for multiple infection was the highest for dual infection with AKAV and BTV (25.6%), followed by triple seropositivity (9.3%). These findings indicated that BTV, AKAV and SBV circulate in camels in Turkey at a relatively high rate, and that an active surveillance program is needed for the management and tracing the dynamics of these infections in the Turkish camel population.

Key words: Akabane; Bluetongue; Schmallenberg; camel; seroprevalence; Turkey

Introduction
Some arthropod-borne (arbo) viruses transmitted by haematophagous flies have become increasingly widespread in recent years with the expansion of habitats of vectors depending on climate change (WHITEHORN and YACOUB, 2019). Akabane virus (AKAV), Bluetongue virus (BTV) and Schmallenberg virus (SBV), classified among non-zoonotic arboviruses, cause reproductive problems such as: early embryonic death, malformations, abortion and infertility in cattle, sheep, goat and camels (DOĞAN, 2018). BTV belongs to the genus Orbivirus in the family Reoviridae. The natural biological vectors for BTV are Culicoides spp. biting midges that transmit the virus among wild and livestock animal species (MELLOR and BOORMAN, 1995; MELLOR et al., 2000; MELLOR and WITMANN, 2002). The virus may cause a severe clinical disease characterized by oedema, haemorrhage and ulceration of mucous membranes in sheep and deer (MELLOR and BOORMAN, 1995; ERTÜRK et al., 2004; BATTEN et al., 2011). BTV infection has also been reported in camelids, such as alpaca and camel (MADANI et al., 2011; WRIGHT, 2014). In Turkey, BTV infections, recognized as serotypes 4, 8, 9 and 16,
Camelus dromaderius), unvaccinated against the investigated viruses, were randomly taken from animals in private ownerships (from volunteer animal owners) from seven provinces (Antalya, Aydın, Çanakkale, Denizli, İzmir, Muğla and Şanlıurfa) in Turkey in 2019 (Fig. 1). The sampled camels were used for wrestling, transport and touristic activities. All blood samples were taken into clot activator vacuum tubes and centrifuged at 5000xg for 10 minutes, then kept at -20 °C until testing.

Serological analysis. Commercial diagnostic ELISA kits were used for detection of BTV (IDVet Bluetongue Competitive ELISA; Pourquier Laboratory, Montpellier, France), AKA V (AKA V Antibody ELISA; JNC Corporation, Tokyo, Japan), and SBV-specific antibodies (INgezim Schmallenberg Compac 2.0, Ingenasa, Spain), as described by the manufacturers. The AKA V ELISA kit microplates were coated with the monoclonal antibody 19B-4 to the Akabane virus G1 protein. The SBV ELISA kit’s conjugate also contains a specific monoclonal antibody for SBV. It was reported by the manufacturer that both the AKA V and SBV antibody kits lack cross-reactivity with Simbu serogroup viruses due to the high specificity of the monoclonal used.

Results and discussion
There was no epidemiological information about BTV in Turkish camels previously reported, although a previous serosurvey showed the presence of antibodies to other orbivirus recognized as EHDV (7.3%) in Turkish camels in Aydın province, Turkey (EROL et al., 2014). In the current study, in the panel of 86 camel sera tested the overall seropositivity rate against BTV in Turkish camels sampled was 53.5%, although the seroprevalence of BTV has been determined to range between 0 to 76.7% in camels worldwide (ERISA et al., 1979; MADANI et al., 2011; TOUIL et al., 2012; YOUSEF et al., 2012; MENTABERRE et al., 2013; MELAKU et al., 2016; SAIDI et al., 2020). However, the BTV antibody prevalence in ruminants in Turkey which vary between 4.3 and 97% (ERTÜRK, 1994; ALBAYRAK and OZAN, 2010; CAITO et al., 2012; YILMAZ and ÖZKUL, 2012; PESTİL, 2014; OIE, 2018). AKAV and SBV belong to the Simbu serogroup of the genus Orthobunyavirus in the family of Peribunyaviridae (WANG et al., 2019). Both AKAV and SBV infections are transmitted to a wide host range, including cattle, sheep, goats, camels, horses and piglets, by biting midges (Culicoides spp.) and/or mosquitoes (Aedes spp. and Culex spp.) (DE REGGE et al., 2012; WERNIKE and BEER, 2017). SBV specific antibodies were also reported in alpaca from South American cameldids (JACK et al., 2012; SCHULZ et al., 2015), in a dog (WENSMAN et al., 2013) and in ruminants, such as cattle, goats, sheep and water buffalo (HOFFMANN et al., 2012; AZKUR et al., 2013). Moreover, SBV causes a mostly subclinical infection in camels (SCHULZ et al., 2015).

Several reports on BTV, AKAV and SBV in ruminants in Turkey have provided more data on the epidemiology of these infections (ÖZGÜNÜLK, 2003; GÜR et al., 2008; KARAOĞLU et al., 2012, 2013; TONBAK et al., 2016; MACUN et al., 2017; DOĞAN, 2018; OIE, 2018), while there are a few reports about some arbovirus infections, such as epizootic haemorragic disease virus (EHDV) and West Nile virus (WNV), and AKAV infections in Turkish camels (EROL et al., 2014 and 2016; KOÇ and EROL, 2017). In this study, the prevalence of BTV, AKAV and SBV infections was investigated serologically in healthy-appearing camels in various parts of Turkey, to provide new insights into the prevalence of BTV, AKAV and SBV infections in Turkish camels to guide future large-scale epidemiological studies.

To the best of our knowledge, this study is the first report on the presence of antibodies to BTV and SBV in Turkish camels.

Materials and methods
Serum samples. A total of 86 sera samples obtained from clinically healthy dromedary camels (Camelus dromaderius), unvaccinated against the investigated viruses, were randomly taken from animals in private ownerships (from volunteer animal owners) from seven provinces (Antalya, Aydın, Çanakkale, Denizli, İzmir, Muğla and Şanlıurfa) in Turkey in 2019 (Fig. 1). The sampled camels were used for wrestling, transport and touristic activities. All blood samples were taken into clot activator vacuum tubes and centrifuged at 5000xg for 10 minutes, then kept at -20 °C until testing.

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in Turkey were associated with the BTV-4 and BTV-8 serotypes (OIE, 2018). Furthermore, the presence of biting midges from the Culicoides spp. such as Culicoides imicola, Culicoides schultzei, Culicoides longipennis, Culicoides circumscriptus, and Culicoides obsoletus was also confirmed in the sampling area (DİK and ERGÜL, 2006; DİK et al., 2012; DOĞAN, 2018).

Moreover, it is thought that transmission of BTV to healthy animals may be caused by direct contact, the transmission of infected blood, using the same water container, and ingestion of infected placenta (MENZIES et al., 2008; LOPEZ et al., 2010), and camels may be a possible risk factor for transmission of BTV infection to other livestock (BÄTEN et al., 2011; MADANI et al., 2011).

In this study, 44 of 86 (51.2%) Turkish camel sera samples tested were positive for AKA V antibodies. In contrast to the current findings for AKA V antibodies in Turkish camels, none of the camels sampled in the Muğla and Aydın provinces in Turkey was positive in terms of AKA V antibodies, as described by elsewhere. It is likely that the disease may be present, but could not be detected because of the low sample size or limited sampling area (KOÇ and EROL, 2017). However, on the basis of previous survey studies for AKA V infection which included the sampling areas, the seroprevalence rate for AKA V infection was reported to be up to 44.9% in ruminants in Turkey (TAYLOR and MELLOR, 1994; ÖZGÜNLÜK, 2003; ÇABALAR and DAĞALP, 2006; KARAOĞLU et al., 2007; KARAOĞLU et al., 2012; PESTİL, 2014; KOÇ and EROL, 2017; ŞEVİK, 2017; DOĞAN, 2018).

The SBV antibody prevalence rate was reported to range from 0.38% to 43.3% in ruminant populations in Turkey (AZKUR et al., 2013; MACUN et al., 2017; BIYIKLI et al., 2017; DOĞAN, 2018), and in previously published studies, the existence of C. obsoletus species as the main vector for SBV was also reported in Turkey (DİK and ERGÜL, 2006; DİK et al., 2012; DOĞAN, 2018). However, there was no information available for SBV in camels in Turkey and although the seroprevalence of SBV in camelids has been reported to be up to 86% worldwide there have been limited reports on the global prevalence of SBV in camelids (JACK et al., 2012; WERNERY et al., 2013; SCHULZ et al., 2015). The antibody prevalence against SBV in dromedary camels was demonstrated in Pakistan (86%), UAE (37%), and Sudan (19%) (WERNERY et al., 2013).
In the current study, the seroprevalence rate in Turkish camels was shown for the first time to be 15.1% (13/86) in the tested animals. Additionally, AKAV antibodies are more prevalent than SBV in the Turkish camels in the current study. The antibody level obtained three weeks after experimental SBV infection in camelids was reported to be very low compared to cattle and sheep (SCHULZ et al., 2015). The detection of low SBV seropositivity in this study may be due to the low antibody level in camels.

The overall seroprevalence of the dual or triple infections examined was 38.4% (33/86) in Turkish camels, while the seropositivity for multiple infection was the highest for dual reactivity against AKAV and BTV (25.6%), followed by triple seropositivity (9.3%). Previous reports argued that the intensity of transmission in arbovirus diseases is dependent on the vectorial capacity and competence of local mosquitoes, and changes in the ecosystem and climate have impacted the transmission of a wide range of some arbovirus diseases worldwide (ROSSATI et al., 2016; SEMENZA and SUK, 2018).

In conclusion, the data revealed that BTV, AKAV and SBV circulate in camels in Turkey, although the current study shows a lower prevalence in terms of SBV compared with AKAV and BTV antibody prevalence in these camels in Turkey. Furthermore, this study provides general epidemiological information about emerging non-zoonotic arbovirus (BTV, AKAV and SBV) infections in Turkish native camels. An active monitoring program is needed to manage and monitor the dynamics of these infections in the Turkish camel population.
Conflict of interest
None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Ethical approval
The study protocol was approved by the Local Animal Ethics Committee of the Institute of Pendik Veterinary Control, Istanbul, Turkey (No.06/2019). Permission for publication was obtained from General Directorate of Food and Control, Ministry of Agriculture and Forestry, Republic of Turkey (09.08.2019/E.2457377).

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