Retrospective Serological Investigation of Epizootic Haemorrhagic Disease Virus in Sheep, Goats and Camels in the Aydin Province, Türkiye

Erol, N.*, Koç, B.T. and Tan, M.T.

Department of Virology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, West Campus, 09016, Isikli, Efeler, Aydin, Türkiye.

* **Corresponding Author:** Prof. Dr. Nural Erol, Department of Virology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, West Campus, 09016, Isikli, Efeler, Aydin, Türkiye. aduvirology@gmail.com , +90-256-600000

ABSTRACT

Epizootic haemorrhagic disease virus (EHDV) causes a systemic viral infection primarily affecting deer but has also led to significant economic losses in cattle. In Türkiye, the infection was first reported in Mugla province in 2007 and has caused fatalities in Aydın province as well. The disease is characterised by systemic blood circulation disorders and death. Data on the epizootiological, clinical, and pathological characteristics and virulence of EHDV in sheep, goats, and camels in Aydın province are very limited. The role of these animals as hosts for the virus is unknown. This study investigated EHDV infection in serum samples collected from 40 goats, 55 sheep, and 68 camels. Samples were tested for EHDV-specific antibodies using a commercial enzyme-linked immunosorbent assay. Antibodies against EHDV were detected in five of the 68 camels (7.3%), seven of the 40 goats (17.5%), and four of the 55 sheep (7.3%). These findings suggest that natural EHDV infection is present and may still be circulating in camels, goats, and sheep in the region. Since EHDV may cause subclinical or atypical symptoms, these animals could play a role in the epidemiology of EHDV infections. Additionally, the virus may occasionally cause outbreaks, resulting in economic losses in the Aegean region. To our knowledge, this is the first report of serological evidence of natural EHDV infection in camels and goats in Türkiye. Results from this preliminary study may be useful in larger future studies aimed at preventing and controlling the infection.

Keywords: Antibody; Camel; Epizootic Haemorrhagic Disease Virus; Goat; Sheep

INTRODUCTION

Epizootic hemorrhagic disease virus (EHDV) is a member of the *Orbivirus* genus within the *Sedoreoviridae* family (1). It has a double-stranded segmented RNA genome. Officially, seven serotypes of EHDV (EHDV-1, -2, -4, -5, -6, -7, and -8) have been recognized (1, 2, 3, 4). However, novel EHDV strains from South Africa, Japan, and China have been proposed as possible serotypes (4). The virus is transmitted between ruminants by midges of the genus *Culicoides spp.* (5).

Epizootic hemorrhagic disease (EHD), sometimes referred to as deer disease, can cause significant mortality events in deer populations but has also been linked to severe clinical symptoms, major loss of productivity, and death in cattle (6). The infection in white-tailed deer leads to serious illness. Although it is thought that EHDV rarely causes disease in domestic ruminants, the infection was first seen in the form of severe clinical symptoms in cattle and outbreaks in Israel and Türkiye in the years 2006-2007 (6, 7, 8).

Data on the prevalence and virulence of the virus and the clinical and pathological findings in camels, goats, and sheep are very limited in literature. It is assumed that sheep are susceptible to EHDV infection but rarely develop clinical symptoms (9, 10). In an outbreak in Israel, Kedmi *et al.* (11) concluded that sheep had no part in the epizootiology

since they could not identify any signs of EHDV infection in sheep. It is not yet clear how goats serve as hosts for the EHDV virus (5). Goats are not considered susceptible to infection. However, some researchers consider that a proportion of goats, as in sheep, may develop a low level of viremia (12). EHDV antibodies have been detected in goats in a few countries under field conditions (2, 13, 14). The infection has been reported in camels as well (15), but there are few studies on the EHDV infection in camels. No serological evidence of EHDV infection in camels or goats has been reported in Türkiye so far, although clinical cases of EHD were reported in sheep flocks around Aydin province (8).

The objective of this study was to investigate the serological presence and distribution of EHDV infection in camels, goats, and sheep using an Enzyme-Linked Immunosorbent Assay (ELISA). Thus, this preliminary study aimed to help understand the epizootiology of EHDV in Turkey. Furthermore, the results from this study are hoped to be useful in larger future studies aimed at preventing and controlling the infection.

MATERIALS AND METHODS

This retrospective study investigated EHDV infection in serum samples from 40 goats, 55 sheep, and 68 camels. The samples were collected from sheep and goats in 2012 and camels in 2011. The tested samples were collected randomly from animals in Aydin province. The 68 blood samples were collected from dromedary camels (*Camelus dromedarius*) that were brought to Incirliova Municipal Slaughterhouse, to the Faculty of Veterinary Clinics of Aydin Adnan Menderes University for medical examination and bred on local farms in Aydin province. The camels brought to slaughter or those to be bred were appeared healthy at the time of blood collection. The animals that were brought to the clinics also did not show any clinical signs of systemic disease. The age of the animals varied between one and 20 years.

The blood samples were collected in polystyrene tubes containing kaolin. Serum was obtained by centrifugation of blood samples collected into tubes containing kaolin and stored at -20°C until testing. All samples were tested with ELISA in the same year of blood collection.

The presence of EHDV-specific antibodies (against VP7) in blood serum samples was tested using a commercially available blocking ELISA (LSIVet, Lissieu, France). The assay was optimized to eliminate cross-reactivity. The test was performed following the manufacturer's instructions. The positive samples were tested in duplicate, in order to confirm the results.

Table 1. Numbers of tested animals and seropositivity rates against EHDV in camel, goat, and sheep

	Tested Animals	EHDV Ab positive	%
Camel	68	5	7.3
Goat	40	7	17.5
Sheep	55	4	7.3
Total	163	16	9.8

RESULTS

In this study, antibodies against EHDV were detected in five of the 68 camels (7.3%), seven of the 40 goats (17.5%), and four of the 55 sheep (7.3%) investigated (Table 1). In total, 16 of the 163 animals were seropositive (9.8%). The findings of this study suggest that natural EHDV infection is present and may be circulating in camels, goats, and sheep in western Türkiye. These animals may contribute in the epidemiology of EHDV infection since EHDV may induce subclinical or unfamiliar symptoms. In addition, the virus may cause outbreaks from time to time, resulting in economic losses in the Aegean region. To our knowledge, this is the first report of serological evidence of natural EHDV infection in camels and goats in Türkiye.

DISCUSSION

There are few reports of EHDV infection in camels, cattle, goats, and sheep in the existing literature. In general, it has been determined that EDHV infection in sheep and goats either doesn't exist or occurs very infrequently (9, 10, 11). It has been observed that giving goats and sheep direct virus inoculation or material containing EHDV did not result in any clinical signs or viremia (9, 11, 16, 17, 18, 19). Temizel *et al.* (7) isolated EHDV in cattle in Türkiye but did not report any evidence of infection in sheep or goats. Kedmi *et al.* (11) claimed that there is no evidence for the involvement of sheep in the epidemiology of cattle.

Eschbaumer *et al.* (16) detected 12 East Frisian sheep with a virulent EHDV-7, isolated from an outbreak in Israel. After the inoculation of sheep with the virus from that outbreak, the viremia was not detectable; the virus could not be

re-isolated. While only a small amount of viral RNA was found in the blood of two sheep, their antibody response after inoculation of sheep with the virus from that outbreak was conflicting. The authors reported that the sheep did not show any symptoms. Therefore, they concluded that the infection did not lead to a productive infection. However, some researchers have isolated EHDV from sheep (8, 20). Also, there is evidence of EHDV infection with clinical symptoms in sheep in Türkiye. Yavru *et al.* (8) isolated EHDV from three sheep showing clinical symptoms in Aydin province. The results of this study and the findings of Yavru *et al.* (8) support the hypothesis that sheep could be natural hosts of EHDV.

Recently, a few studies have been published about the sero-prevalance of EHDV infection in cattle, goats, and sheep (21, 22). In a study in Zimbabwe, the median seroprevalences of EHDV antibodies in cattle and sheep were found to be 62% and 0%, respectively (21). No EHDV antibodies were detected in an epidemiological study in goats and sheep in Kazakhstan (22). Hampy (23) detected antibodies against EHDV in three sheep in Texas, USA. Odiawa *et al.* (13) found the highest seropositivity in goats and sheep in the American state of Georgia. They detected 29% of sheep (n=286) and 7% of goats (n=433) were seropositive for EHDV. Cêtre-Sossah *et al.* (2) investigated 276 cattle, 142 sheep, and 71 goats on Reunion Island. They observed that the EHDV seroprevalence rate in cattle was 63.77%, 5.63% in goats, and 3.70% in sheep. They suggested that EHD widely occurs in cattle rather than in goats and sheep. Mahmoud *et al.* (24) studied the seroprevalence of EHDV infection in goats, cattle, and sheep in Libya. They found it to be 4% as the overall seroprevalence rate (small ruminants and cattle) of EHDV. Of the 555 sheep tested, 32 were found positive; none of the 135 goats were positive; and only 1 out of 165 cattle was found to be positive (24). In a field study conducted in Indonesia, antibodies against EHDV serotype 5 using seroneutralization were found to be 24% (150/620), 1.8% (1/62), and 0% (0/20) in cattle, goats, and sheep, respectively (14). In line with the previous study, we discovered a high seropositivity rate in goats. It is possible that goats are susceptible to EHDV and could act as natural reservoirs. Further studies are needed to understand the susceptibility and the role of goats as hosts for EHDV.

There are a few reports of various serotypes of epizootic hemorrhagic disease virus infecting camels (25, 26). Wernery *et al.* (26) showed that 29% of dromedary camels from the United Arab Emirates have antibodies to EHDV. Cossedou *et al.* (25) observed 63 out of 86 camels (73%) seropositivity for EHDV in Mauritania. This seropositivity rate was the highest in the world. However, EHDV infection was not found in camels in seroepidemiological studies conducted in Tunisia and Morocco (15, 27, 28). There are no detailed studies on the clinical appearance of EHDV in camels. In this study, the rate of EHDV-specific antibodies was detected to be 7.8% in camels. This finding supports reports that camels may be natural hosts of EHDV infection and could play a role in epizootiology.

The Aydin locality is one of the regions where vector diseases such as akabane, bluetongue, and ephemeral fever are frequently seen. This region has suitable ecological and geographical conditions for the biological circulation of blood-sucking mosquitoes as biological vectors, especially *Culicoides spp.* The samples investigated in the study were collected during the period when EHDV infection was clinically prevalent. In the study, although EHDV infection seropositivity was observed in camels, goats, and sheep, clinical findings were found only in sheep. In contrast to previous studies, this study revealed the infection with solid evidence of the serologic presence of EHDV in camels, goats, and sheep. The clinical findings in sheep in the Aydin region (8) supported the results obtained in this study. The infection in these animals may depend on the serotype or topotype of the virus and epizootiological factors such as animal species, breed, geographic location, climate, etc. in the region. Therefore, further arthropodological, epizootiological, and molecular virological studies should be performed in the region. In addition, experimental pathogenesis and clinical studies with serotypes and topotypes of regional EHDV need to be carried out in these animals through further investigations.

CONCLUSIONS

EHDV might be causing sporadic atypical symptoms and subclinical infections that result in complications in the differential diagnosis of various viral infections. Thus, periodic controls should be carried out on camels, goats, and sheep and prevention and control strategies should be developed accordingly. Especially in regions such as Aydın, where the average temperature and humidity remain relatively high

even in winter and create an environment that favors the growth of bloodsucking flies, it is necessary to increase the control of stinging flies and research on EHDV infections in different animal species.

The results obtained in this study demonstrated the presence of EHDV in camels, goats, and sheep in the Aydin region. However, it is still unquestionably unknown if EHDV triggers what form of clinical symptoms and pathogenesis in camels, goats, and sheep. Therefore, these species should not be ignored in the epidemiology of EHDV. Further research, including molecular detection analysis and next-generation sequencing, is required to understand the role of sheep, goats, and camels in the epidemiology of EHDV.

ACKNOWLEDGMENTS

Ethical statement

Aydın Adnan Menderes University Animal Experiments Local Ethics Committee approved the study with Decision No. 050.04/2011/123.

Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Walker, P. J., Siddell, S. G., Lefkowitz, E. J., Mushegian, A. R., Adriaenssens, E. M., Alfenas-Zerbini, P., Dempsey, D. M., Dutilh, B. E., García, M. L., Hendrickson, R. C., Junglen, S., Krupovic, M., Kuhn, J. H., Lambert, A. J., Łobocka, M., Oksanen, H. M., Orton, R. J., Robertson, D. L., Rubino, L., Sabanadzovic, S., Simmonds, P., Smith, D. B., Suzuki, N., Van Doorslaer, K., Vandamme, A. M., Varsani, A. and Zerbini, F. M.: Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses. Arch. Virol. 1-12, 2022.
2. Cêtre-Sossah, C., Roger, M., Sailleau, C., Rieau, L., Zientara, S., Bréard, E., et al.: Epizootic haemorrhagic disease virus in Reunion Island: Evidence for the circulation of a new serotype and associated risk factors. Vet. Microbiol. 170:383-390, 2014.
3. Maclachlan, N. J., Zientara, S., Savini, G. and Daniels, P. W.: Epizootic haemorrhagic disease. Rev. Sci. Tech. Off. Int. Epiz. 34:341-351, 2015.
4. Yang, H., Li, Z., Wang, J., Li, Z., Yang, Z., Liao, D., Zhu, J. and Li, H.: Novel serotype of epizootic hemorrhagic disease virus, China. Emerg. Infect. Dis. 26:3081-3083, 2020.
5. EFSA (European Food Safety Authority): Scientific Opinion on Epizootic Hemorrhagic Disease. EFSA J. 7:1418, 2009.
6. Kedmi, M., Van Straten, M., Ezra, E., Galon, N. and Klement, E.: Assessment of the productivity effects associated with epizootic hemorrhagic disease in dairy herds. J. Dairy Sci. 93:2486-2495, 2010.
7. Temizel, E. M., Yesilbag, K., Batten, C., Senturk, S., Maan, N. S., Mertens, P. P. C. and Batmaz, H.: Epizootic hemorrhagic disease in cattle, Western Turkey. Emerg. Infect. Dis. 15:317-319, 2009.
8. Yavru, S., Erol, N., Avci, O., Esin, E. and Pasa, S.: Isolation of epizootic hemorrhagic disease virus from sheep in Western Turkey. Rev. Méd. Vét. 164:20-624, 2014.
9. Gibbs, E. P. J. and Lawman, M. P. J.: Infection of British deer and farm animals with epizootic haemorrhagic disease of deer virus. J. Comp. Pathol. 87:335-342, 1977.
10. Uren, M. F.: Clinical and pathological responses of sheep and cattle to experimental infection with five different viruses of the epizootic haemorrhagic disease of deer serogroup. Aust. Vet. J. 63:199-201, 1986.
11. Kedmi, M., Levi, S., Galon, N., Bomborov, V., Yadin, H., Batten, C. and Klement, E.: No evidence for involvement of sheep in the epidemiology of cattle virulent epizootic hemorrhagic disease virus. Vet. Microbiol. 148:408-412, 2011.
12. Al-Busaidy, S. M. and Mellor, P. S.: Isolation and identification of arboviruses from the Sultanate of Oman. Epidemiol. Infect. 106:403-413, 1991.
13. Odiawa, G., Blue, J. L., Tyler, D. E. and Shotts, E. B.: Bluetongue and epizootic hemorrhagic disease in ruminants in Georgia: Survey by serotest and virologic isolation. Am. J. Vet. Res. 46:2193-2196, 1985.
14. Sendow, I., Daniels, P. W., Cybinski, D. H., Young, P. L. and Ronohardjo, P.: Antibodies against certain bluetongue and epizootic haemorrhagic disease viral serotypes in Indonesian ruminants. Vet. Microbiol. 28:111-118, 1991.
15. Touil, N., Cherkaoui, Z., Lmrabih, Z., Loutfi, C., Harif, B. and El Harrak, M.: Emerging viral diseases in dromedary camels in Southern Morocco. Transbound. Emerg. Dis. 59:177-182, 2012.
16. Eschbaumer, M., Wernike, K., Batten, C. A., Savini, G., Edwards, L., Di Gennaro, A., Teodori, L., Oura, C. A. L., Beer, M. and Hoffmann, B.: Epizootic hemorrhagic disease virus serotype 7 in European cattle and sheep: Diagnostic considerations and effect of previous BTV exposure. Vet. Microbiol. 159:298-306, 2012.
17. Fletch, A. L. and Karstad, L. H.: Studies on the pathogenesis of experimental epizootic hemorrhagic disease of white-tailed deer. Can. J. Comp. Med. 35:224-229, 1971.
18. Foster, N. M., Breckon, R. D., Luedke, A. J. and Jones, R.: Transmission of two strains of epizootic hemorrhagic disease virus in deer by Culicoides variipennis. J. Wildl. Dis. 13:9-16, 1977.
19. Tomori, O.: Bluetongue and related viruses in Nigeria: Experimental infection of West African dwarf sheep with Nigerian strains of the viruses of epizootic haemorrhagic disease of deer and bluetongue. Vet. Microbiol. 5:177-185, 1980.
20. Noon, T. H., Wesche, S. L., Cagle, D., Mead, D. G., Bicknell, E. J., Bradley, G. A., Riplog-Peterson, S., Edsall, D. and Reggiardo, C.: Hemorrhagic disease in bighorn sheep in Arizona. J. Wildl. Dis. 38:172-176, 2002.
21. Gordon, S. J. G., Bolwell, C., Rogers, C. W., Musuka, G., Kelly, P., Guthrie, A., Mellor, P. S. and Hamblin, C.: A serosurvey of bluetongue and epizootic haemorrhagic disease in a convenience sample of sheep and cattle herds in Zimbabwe. Onderstepoort J. Vet. Res. 84:1-5, 2017.
22. Lundervold, M., Milner-Gulland, E. J., O'Callaghan, C. J., Ham-

blin, C., Corteyn, A. and Macmillan, A. P.: A serological survey of ruminant livestock in Kazakhstan during post-Soviet transitions in farming and disease control. Acta Vet. Scand. 45:211-224, 2004.
23. Hampy, B.: Serological studies on sympatric Barbary sheep and mule deer from Palo Duro Canyon, Texas. J. Wildl. Dis. 15:443-446, 1979.
24. Mahmoud, A., Danzetta, M. L., di Sabatino, D., Spedicato, M., Dayhum, A., Tolari, F., Forzan, M., Mazzei, M. and Savini, G.: First seroprevalence investigation of epizootic haemorrhagic disease virus in Libya. Open Vet. J. 11:301-308, 2021.
25. Cosseddu, G. M., Doumbia, B., Scacchia, M., Pinoni, C., Di Provvido, A., Polci, A., Isselmou, K., Di Gennaro, A., Spedicato, M., Carmine, I., Savini, G., Dondona, A. C., Iapaolo, F., Valleriani, F., El Mamy, A. B., Barry, Y. and Monaco, F.: Sero-surveillance of emerging viral diseases in camels and cattle in Nouakchott, Mauritania: An abattoir study. Trop. Anim. Health Prod. 53:195, 2021.
26. Wernery, U., Thomas, R., Raghavan, R. and Syriac, G.: Serological evidence of epizootic haemorrhagic disease and Schmallenberg virus in dromedaries. J. Camel Pract. Res. 20:135-137, 2013.
27. Hassine, T. B., Amdouni, J., Monaco, F., Savini, G., Sghaier, S., Selimen, I. B., Chandoul, W., Hamida, K. B. and Hammami, S.: Emerging vector-borne diseases in dromedaries in Tunisia: West Nile, bluetongue, epizootic haemorrhagic disease and Rift Valley fever. Onderstepoort J. Vet. Res. 84:1316, 2017.
28. Mejri, S., Dhaou, S. B., Jemli, M., Bréard, E., Sailleau, C., Sghaier, S., Zouari, M., Lorusso, A., Savini, G., Zientara, S. and Hammami, S.: Epizootic haemorrhagic disease virus circulation in Tunisia. Vet. Ital. 54:87-90, 2018.