Bulgarian Journal of Veterinary Medicine, 2023, **26**, No 2, 202–207
ISSN 1311-1477; DOI: 10.15547/bjvm.2021-0019

Original article

SEROPREVALENCE OF CRIMEAN-CONGO HAEMORRHAGIC FEVER IN SHEEP AND GOATS IN IRAQ

M. A. S. ALTALIBY, S. A. ESMAEEL & KH. J. HUSSAIN

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Summary

Altaliby, M. A. S., S. A. Esmaeel & Kh. J. Hussain, 2023. Seroprevalence of Crimean-Congo haemorrhagic fever in sheep and goats in Iraq. *Bulg. J. Vet. Med.,* **26**, No 2, 202–207.

Crimean-Congo haemorrhagic fever (CCHF) causes haemorrhagic disease in human beings with high mortality rate and it is typically asymptomatic in animals. Data for livestock exposure to CCHF and its risk in Mosul, Iraq are scarce. Therefore, the present study was designed to investigate the seroprevalence of CCHF in sheep and goats and some risk factors for the disease. From April 2019 to October 2019, two hundred blood samples (from 120 sheep and 80 goats) were collected from privately-owned farms located in different parts of Mosul city. Specific IgG antibodies against CCHF virus were examined using indirect enzyme linked immunosorbent assay kit (I-ELISA). The results revealed an overall prevalence rate of 14%, 19.16% and 6.25% in sheep and goats respectively. Significantly ($P \leq 0.05$) higher prevalence was recorded in imported sheep (23.3%) compared with the local sheep breed (6.66%), as well significant ($P \leq 0.05$) variations of risk between tick-infested and tick-free animals. CCHF detected in sheep and goats constitutes a risk to public health in Mosul city, Iraq. More studies are recommended to further investigate the disease in other animal species.

Key words: Crimean-Congo haemorrhagic fever, I-ELISA, Iraq, goats, Mosul, sheep

INTRODUCTION

Crimean-Congo haemorrhagic fever virus (CCHFV), genus *Orthonairovirus*, family *Nairoviridae* of the order Bunyavirales (Abudurexiti *et al*., 2019) is enzootic in the southern part of Europe (Balkans), Turkey, Russia, and Middle East and North Africa (MENA) countries, as well as in central Asia and western part of China (Appannanavar & Mishra, 2011; Swanepoel & Paweska, 2011). The spread of the disease is dependent on the presence of vector; ticks of the genus *Hyalomma* (Bell-Sakyi *et al*., 2012). *Hyalomma* ticks infect a wide range of different animal species, such as sheep, goats, cattle, and wild animals. The distribution of infected ticks to various unaffected regions accelerates the proliferation of the virus, but viraemia in livestock is described as short-lived, and not intense, yet with a crucial role in the life cycle of ticks, transmitting and propagation of the

virus. In animals, no clinical symptoms are manifested, so CCHF infections have no adverse economic impact on the livestock production sector (Swanepoel & Paweska, 2011). Unlike animals, infection of humans can lead to debilitating illness. The first report of CCHF in Iraq was in 1979 primarily in Baghdad (Al-Tikriti *et al.,* 1981). Currently, there is no available CCHF vaccine and the treatment of the disease is in accordance with the observed signs (Mertens *et al.,* 2013).

In terms of identifying the agent, only one virus serotype has been found so far. CCHFV has morphological and physicochemical properties common to the family *Nairoviridae*. The virus has a single-stranded, negative-sense RNA and could be isolated from serum or plasma samples obtained during the febrile or viraemic stage of infection, or sourced from the liver of infected animals by inoculation of several tissue cultures (Swanepoel & Paweska, 2011). To identify and characterise the virus, traditional and real-time reverse transcriptase-polymerase chain reaction (PCR) may be employed. Currently, there are commercial CCHFV kits for detection of IgM or IgG by enzyme linked immunosorbent assay (ELISA) or immunofluorescence (IFA). The benefit of ELISA test is that it facilitates the analysis of different animal species with high specificity and sensitivity, as well as the determination of CCHF-affected environments, as antibody prevalence in animals can reliably indicate local virus circulation (Dowell *et al.,* 2012).

The role of domestic ruminants in CCHF zoonosis in Mosul, Iraq, has not been adequately investigated despite reports of a CCHF epidemic in the neighbouring countries, namely the Republic of Turkey and Iran (Ergonul, 2006; Mostafavi *et al.*, 2013).

This study was motivated by the urgent need to assess the current status of CCHF among ruminants in Mosul. It was carried out to investigate the seroprevalence of CCHF antibodies in sheep and goats from different areas in Mosul by ELISA, and to evaluate some risk factors for these animals and public health.

MATERIALS AND METHODS

Ethical approval

The blood samples were obtained in accordance with the recommended standard sample collection procedure, which ensured that animals were not subjected to any stress or harmed in any way.

Animals and collection of samples

From April to October 2019, two hundred serum samples were collected from apparently healthy adult animals (120 sheep and 80 goats) using the formula for known populations (Thrusfield, 2007), of both genders, aged 1.5–3 years. They were imported (from Iran, Turkey and Syria) and from a local breed, belonging to privately-owned farms from different areas in Mosul, Iraq. An amount of 10 mL of blood was drawn from the jugular vein of each animal using a sterile syringe into a sterile vacutainer tube without anticoagulant for serum, and transported in cool conditions to the laboratory of the Veterinary Medicine College, University of Mosul, and stored at –20 °C until used (Stockham & Scott, 2010).

Indirect enzyme-linked immunosorbent assay (I-ELISA)

Double antigen ELISA kit (ID Vet, France) for the detection of antibodies against CCHFV in the serum of sheep and goats was used according to the tech-

niques described by the manufacturer. The absorbance of samples in the ELISA plate was recorded at 450 nm employing Bio-Tek EL-800 micro plate reader. Optical density (OD) values were determined from the readings and uploaded to a Microsoft Excel spreadsheet. Samples presenting percentage (SP %) were calculated using the formula provided by the manufacturer, and samples were differentiated as positive or negative:

SP% = (mean OD of sample/mean OD of positive control) ×100.

Results with SP ≤ 30 were interpreted as negative, and those with SP > 30: as positive.

Statistical analysis

Chi-square statistics (IBM SPSS Statistics version 19) was utilised to determine how infection and the variables of the study were associated, where P value of 0.05 was considered significant. Statistical analysis for risk factors data was performed using Epi-Info TM 7.

RESULTS

Serological evidence for CCHF infection was found in 28 (14%) of the tested 200 serum samples, which included 23 of 120 sheep (19.16%) and 5 of 80 goats(6.25%), which were CCHFV IgG antibody-positive by indirect ELISA (Table 1).

The prevalence of CCHF was also significantly increased among sheep imported from Iran, Turkey and Syria (23.3% of 90 animals) compared to the local sheep breed (6.66% of 30 sheep (P≤0.05). All goats in this study were from a local breed (Table 2).

Heavily tick-infested animals (139 sheep and goats) showed significant (P≤0.05) variations in the prevalence of CCHF in comparison with the 61 tick-free animals (17.2% vs 6.5% respectively, Table 2).

Table 1. Seroprevalence of anti-CCHFV IgG antibody in sheep and goats

Animals	Number of animals	Number (%) of CCHFV-positive	Number (%) of CCHFV-negative
Sheep	120	23 (19.16%)	97 (80.83%)
Goats	80	5 (6.25%)	75 (93.75%)
Total	200	28 (14.00%)	172 (86.00%)

Table 2. Seroprevalence rate of anti-CCHFV IgG antibody-based on animals' breed and tick infestation

Animals	Number of animals	Number (%) of CCHFV-positive
Imported sheep	90	21 (23.3%)*
Local sheep breed	30	2 (6.66%)
Heavy tick-infested sheep and goats	139	24 (17.2%)**
Tick free sheep and goats	61	4 (6.5%)

* P≤ 0.05 between imported and local sheep; ** P≤ 0.05 between tick-infested and tick-free sheep and goats.

DISCUSSION

CCHF is a zoonotic viral disease, asymptomatic in infected animals, with a public health significance. Viraemic livestock, especially cattle, sheep and goats may be good sources of direct transmission to humans and other animals (Kagunyu & Wanjohi, 2014). Laboratory testing has been significantly beneficial in detecting CCHFV antibodies in domestic animals and making available initial evidence of viral circulation and localisation as well as evaluation of risk for animal herders and other human beings (Ceianum *et al.,* 2012).

The seroprevalence of CCHF infection in the current study revealed that the rate of detection of IgG antibodies in serum samples of sheep and goats was 14%, which included 19.16% of sheep and 6.25% of goats as determined by using indirect ELISA. The results of the study differ from those of studies conducted in other countries. In Iran, for example, recorded prevalence rates in more than one study conducted on sheep ranged from 12.6% to 77.5%, by using IFA and ELISA tests (Saidi *et al*., 1975; Telmadarraiy *et al.,* 2010; Chinikar *et al.,* 2012). Another study in Iran conducted on goats revealed that the prevalence rate of the disease ranged from 9.5% to 40% (Khan *et al.,* 1997; Telmadarraiy *et al.,* 2010; Chinikar *et al.,* 2012). In the Republic of Turkey, a prevalence study conducted on sheep and goats revealed rates of 31.8% and 66% respectively using ELISA (Tuncerm *et al.,* 2014). The variances between the results of different studies in different countries may be due to the endemic nature of CCHF and the important role of the tick population, as well as the differences between specificity and sensitivity of laboratory tests used for detection of antibodies (Abdiyeva *et al.,* 2019). The results of some studies in Basrah, Iraq and in the Sultanate of Oman agreed with the results of our study as they reported the prevalence of the disease in 20% of sheep (Al-Yabis *et al.,* 2005) and 4.8% in goats (Hussain Body *et al.,* 2019).

The results also showed that the imported sheep had a significantly higher rate of seroprevalence of CCHF IgG compared with local breeds. The reason may be the fact that the sources of imported animal were Iran and the Republic of Turkey, considered CCHF-endemic countries. Therefore, these imported animals have been exposed to infection and had antibody titres, so gave positive test results.

In this study, a statistically significantly ($P \leq 0.05$) higher prevalence was recorded in more heavily tick-infested animals than in tick-free animals. This result may be due to the important role of tick vectors in the transmission of CCHFV (Bell-Sakyi *et al*., 2012).

CONCLUSION

This study is the first epidemiological investigation of CCHF prevalence in small ruminants in the Mosul city, northern Iraq. The study results showed high prevalence of CCHF in sheep and goats of this region and recognised some risk factors related to CCHF infection. Further studies are needed to acquire better understanding and to give more consideration to the disease.

REFERENCES

Abdiyeva, K., N. Turebekov, A. Dmitrovsky, N. Tukhanova, A. Shin, L. Yeraliyeva, N. Heinrich, M. Hoelscher, R. Yegemberdiyeva, Z. Shapiyeva, Z. Kachiyeva, A. Zhalmagambetova, J. Montag, G. Dobler, J. Zinner, E. Wagner, S. Frey & S. Ess-

bauer, 2019. Seroepidemiological and molecular investigations of infections with Crimean-Congo haemorrhagic fever virus in Kazakhstan. *International Journal of Infectious Diseases*, **78**, 121–127.

Abudurexiti, A., S. Adkins, D. Alioto, S. V. Alkhovsky, T. Avšič-Županc, M. J. Ballinger & J. H. Kuhn, 2019. Taxonomy of the order *Bunyavirales*: Update, 2019. *Archives of Virology*, **164**, 1949–1965.

Al-Tikriti, S. K., F. Al-Ani, F. J. Jurji, H. Tantawi, M. Al-Moslih, N. Al-Janabi, M. I. A. Mahmud, A. Al-Bana, H. Habib, H. Al-Munthri, S. H. Al-Janabi, K. Al-Jawahry, M. Yonan, F. Hassan & D. I. H. Simpson, 1981. Congo/Crimean haemorrhagic fever in Iraq. *Bulletin of the World Health Organization*, **59**, 85–90.

Al-Yabis, A. S., A. A. K. Al-Thamery & H. J. Hasony, 2005. Seroepidemiology of Crimean-Congo haemorrhagic fever in the rural community of Basrah. *The Medical Journal of Basrah University*, **23**, 30–35.

Appannanavar, S. B. & B. Mishra , 2011. An update on Crimean-Congo hemorrhagic fever. *Journal of Global Infectious Diseases*, **3**, 285–292.

Bell-Sakyi, L., D. Kohl, D. A. Bente, & J. F. Fazakerley, 2012. Tick cell lines for the study of Crimean-Congo hemorrhagic fever virus and other arboviruses. *Vector-Borne and Zoonotic Diseases*, **12**, 769–781.

Ceianu, C. S., R. I. Panculescu-Gatej, D. Coudrier & M. Bouloy, 2012. First serologic evidence for the circulation of Crimean-Congo hemorrhagic fever virus in Romania. *Vector-Borne and Zoonotic Diseases,* **12**, 718–721.

Chinikar, S., S. M. Ghiasi, S. Naddaf, N. Piazak, M. Moradi, M. R. Razavi, N. Afzali, A. Haeri, K. Mostafavizadeh, B. Ataei, M. Khalilifard-Brojeni, S. M. Husseini & M. Bouloy, 2012. Serological evaluation of Crimean-Congo hemorrhagic fever in humans with high-risk professions living in enzootic regions of Isfahan province of Iran and genetic analysis of circulating strains. *Vector-Borne and Zoonotic Diseases*, **12**, 733–738.

Dowell, S. D., K. S. Richards, V. A. Graham, J. Chamberlain & R. Hewson, 2012. Development of an indirect ELISA method for the parallel measurement of IgG and IgM antibodies against Crimean-Congo haemorrhagic fever (CCHF) virus using recombinant nucleoprotein as antigen. *Journal of Virological Methods,* **179**, 335–341.

Ergonul, O., 2006. Crimean-Congo haemorrhagic fever. *Lancet Infectious Diseases*, **6**, 203–214.

Hussain Body, M. H., A. H. ALrawahi, H. M. Hussain, M. S. Ahmed, S. S. ALHabsi, S. AL-Maklady, M. Al-Maewaly & S. Rajamony, 2016. Cross-sectional survey of Crimean-Congo hemorrhagic fever virus in the sultanate of Oman. *Journal of Veterinary Medicine and Animal Health,* **8**, 44–49.

Kagunyu, A. W. & J. Wanjohi, 2014. Camel rearing replacing cattle production among the Borana community in Isiolo County of Northern Kenya, as climate variability bites. *Pastoralism*, **4**, 1–5.

Khan, A. S., G. O. Maupin, P. E. Rollin, A. M. Noor, H. H. Shurie, A. G. Shalabi, S. Wasef, Y. M. A. Haddad, R. Sadek, I. Khaled, C. J. Peters & T. G. Ksiazek, 1997. An outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates, 1994–1995. *The American Journal of Tropical Medicine and Hygiene*, **57**, 519–525.

Mertens, M., K. Schmidt, A. Ozkul & M. H. Groschup, 2013. The impact of Crimean-Congo hemorrhagic fever virus on public health. *Antiviral Research,* **98**, 248–260.

Mostafavi, E., A. Haghdoost, S. Khakifirouz & S. Chinikar, 2013. Spatial analysis of Crimean Congo hemorrhagic fever in Iran. *The American Journal of Tropical Medicine and Hygiene,* **89**, 1135–1141.

Saidi, S., J. Casals, M. A. Faghih & A. A. Faghih, 1975. Crimean hemorrhagic fever-Congo (CHF-C) virus antibodies in man,

and domestic and small mammals, in Iran. *The American Journal of Tropical Medicine and Hygiene*, **24**, 353–357.

Stockham, S. L. & M. A. Scott, 2008. Fundamentals of Veterinary Clinical Pathology. 2nd edn, Wiley-Blackwell, p. 17.

Swanepoel, R. & J. T. Paweska, 2011. Crimean-Congo hemorrhagic fever. In*: Oxford Textbook of Zoonosis: Biology, Clinical Practise and Public Health Control*, 2nd edn, eds S. R. Palmer, L. Soulsby, P. R. Torgerson & D. W. G. Brown, Oxford University Press, UK, pp. 287–293.

Telmadarraiy, Z., S. M. Ghiasi, M. Moradi, H. Vatandoost, M. R. Eshraghian, F. Faghihi, Z. Zabiollahet, H. Ali & Ch. Sadegh, 2010. A survey of Crimean-Congo haemorrhagic fever in livestock and ticks in Ardabil Province, Iran during 2004–2005. *Scandinavian Journal of Infectious Diseases*, **42**, 137–141.

Thrusfield, M., 2007.Veterinary Epidemiology, 3rd edn, Blackwell Science Ltd., Oxford, p. 624.

Tuncer, P., K. Yesilbag, G. Alpay, E. Dincer, A.O. Girisgin & L. Aydin, 2014. Crimean-Congo hemorrhagic fever infection in domestic animals in the Marmara region, Western Turkey. *Ankara Üniversitesi Veteriner Fakültesi Dergisi,* **61**, 49–53.

Paper received 18.02.2021; accepted for publication 17.04.2021

Correspondence:

Khder Jassiem Hussain,
Department of Internal and Preventive Medicine, College of Veterinary Medicine,
University of Mosul, Mosul, Iraq,
e-mail: khderhussain@uomosul.edu.iq,
https://orcid.org/0000-0003-4436-6760