Full Length Research Paper

Cross-sectional survey of Crimean-Congo hemorrhagic fever virus in the sultanate of Oman

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne hemorrhagic disease caused primarily by a zoonotic virus. The disease may cause a fatal hemorrhagic illness in human; however, CCHF is asymptomatic in infected animals. After the recent reports of CCHF in Oman, a cross-sectional survey was conducted during 2013 to 2014 to study the prevalence in livestock and ticks in order to assess the potential risk of emergence. 1289 serum samples were collected from different domestic animals, and tested by two types of modified ELISA (Indirect and competitive ELISA) for detection of CCHF specific immunoglobulin G (IgG). A prevalence of 7.1% positive serum samples was recorded. Highest individual prevalence was recorded in cattle (17.5%) followed by camels (15.7 %), goat (4.8%) and sheep (4.3%). Moreover, 174 ticks were also collected from 179 location. In total, 9 tick samples were found positive for CCHF virus by Antigen-capture ELISA test and RT-PCR which represents 5.1%. Moreover, the concomitant findings of positive sera for animals and detection of CCHF virus was reverse transcription polymerase chain reaction (RT-PCR). This study aims to estimate the disease distribution in livestock in Oman, identify areas under high risk, and contemplate a comprehensive control strategy.

Key words: Crimean Congo hemorrhagic fever, virus, survey, livestock, ticks, Oman.

INTRODUCTION

Crimean-Congo Hemorrhagic Fever Virus (CCHFV) belongs to the Nairovirus genus of the Bunyaviridae family. It causes a zoonotic disease in many countries of Africa, Asia, Europe and Middle east (Appannanavar and Mishar, 2011). The distribution of CCHFV coincides with the distribution of the main vectors, ticks of the genus *Hyalomma.* CCHF is asymptomatic in infected animals but case fatality can reach up to 80% in humans (Watts et al., 1989; Appannanavar and Mishar, 2011; Kamboj and Pathak, 2013).

Ticks are the major vectors for the transmission of the disease; however, secondary cases due to nosocomial contamination and direction contamination are frequently reported among health care workers and slaughterhouse

personals (Maltezou and Papa, 2010). The disease was first recognized in the Crimean region of Russia in the 1940s, and is now reported in many regions of the word; Africa, Europe and Asia (Ergunay et al., 2010).

In the Sultanate of Oman, CCHF was first reported and confirmed with clinical disease in 4 persons during 1995 to 1996 (Scrimgeour, 1996), from 1997 to 2010 no cases were reported. From 2011 onwards, the cases has been rising up, one case was recorded in 2011, 3 cases in 2012, 10 cases in 2013 and 16 cases in 2014 (Ministry of Health, Sultanate of Oman).

Keeping in view the public health related importance of disease, this study aims to estimate the prevalence, and to build a disease distribution of CCHF in livestock in Oman in order to identify areas under high risk, and also to contemplate a comprehensive control strategy, to assess the prevalence of CCHF in Oman, a convenience sample (animal sera and ticks) was collected. Sera were first examined by modified indirect ELISA. Positive samples were confirmed by competitive ELISA (C-ELISA) for immunoglobulin G (IgG) to CCHF virus (serum). Ticks were tested by antigen-capture ELISA and RT-PCR.

MATERIALS AND METHODS

Sample collection

Animal sera collection

Field serum sampling was carried out throughout the Sultanate of Oman. Local coordinates of each sampling site were obtained using a GPS Garmin, USA. A total of 1289 serum samples collected from 217 cattles, 102 camels, 537 goats, and 433 sheeps were tested for detection of antibodies against CCHF.

Tick collection

A total of 174 ticks were collected (16 from cattle, 92 camels, 26 goats and 40 sheep). Each randomly selected animal was screened for the presence of ticks and if present, the ticks were removed using forceps. From each animal, 2 tick samples were collected, one was preserved in 75% ethanol for species identification in future studies, other sample was kept alive in dry vial and stored at -80°Č, and later was tested for CCHF antigen through commercial ELISA test (Vectorbest, Novosibirsk, Russia) following the manufacture protocol, serum from animals with positive ticks collected and tested for CCHF antibodies by indirect and competitive ELISA.

Modified indirect ELISA test

Human IgG ELISA (Vector-Best) which was initially developed for human use was modified in the authors institute for detection of Ab in cattle, Ovine, Caprine and Camel through replacing the anti-human IgG horseradish peroxidase labeled conjugate by anti-ruminant IgG horseradish peroxidase labeled conjugate (LSIVet). To reduce the nonspecific binding, the optimal working dilution of the conjugated were determined by chessboard titration 1/32, the blocking diluents buffer used for the conjugate contains 0.01 M phosphate Buffer Saline PH 7.4 +/-o.20 plus o.05%(v/v)Tween 20 plus 5% skimmed milk powder. The cut-off value was determined by subtracting OD value of negative from the average value of positive control, samples were considered positive if the OD value was greater than or equal to this cut-off value.

Competitive ELISA test

C-ELISA (Vector-Best Kit) for detection of IgG against CCHFV in human serum was designed based on competition between positive human sera supply in the kit and the animals' sera to be tested. In summaries, the animal sera were firstly added to the coated CCHFV plate and then human positive sera supply in the kit was added then the procedure completed as manufactured. Presentation and interpretation of the results; Expressed as percentage of inhibition calculated according to formula % competition= $OD_{specimen}/OD_{negative\ control}$ x100.

RT-PCR

Suspected ticks were rinsed and homogenized, and centrifuged at 1000 rpm, the supernatant were taken for PCR. The RNA was extracted using Trizol Reagent for homogenization, chloroform for the phase separation and the aqueous phase which constitutes RNA was taken, Isopropyl alcohol was used for precipitation of the RNA, and 75% of ethyl alcohol was used to purify the RNA. The kit used was Shanghai ZJ Biotech, Co, Ltd China, using ABI prism 7500 Real time PCR system (Applied Biosystems, CA, USA)

RESULTS

Serum samples collected from animals with ticks found positive to CCHFV were screened by C-ELISA for CCHF specific antibodies, showing inhibition percent ranging from 80 to 90%. The study considered this serum as positive control, and used to evaluate the modified indirect ELISA test. The inhibition percentage of the negative serum from tick free animals present at livestock research center was ≤ 20. Positive and negative serum by Indirect ELISA was further confirmed by C-ELISA, and these samples were considered as positive and negative control and there were agreement between the values of the two tests.

Serological evidence for CCHFV infection was present in 92 samples (7.1%) of 1289 serum samples examined, location of the study areas is shown on the GIS map in Figure 1. Overall, 38 (17.5%) of 217 cattle, 16 (15.5%) of 102 camel, 26 (4.8%) of 537 goat and 12 (4.3%) of 433 sheep were CCHF IgG antibody-positive.

In total, 174 ticks collected from 16 cattle, 92 camels, 26 goats and 40 sheep during the field surveillance were tested by antigen capture ELISA test, and confirm by RT-PCR (Figure 2). Nine (5.1%) tick samples were found positive (Figure 3). Animal species related distribution of positive ticks was 5 (5.4%) in camels, 2 (12.5%) in cattle, 1 (2.5%) in sheep and 1 (3.8) in goats.

DISCUSSION

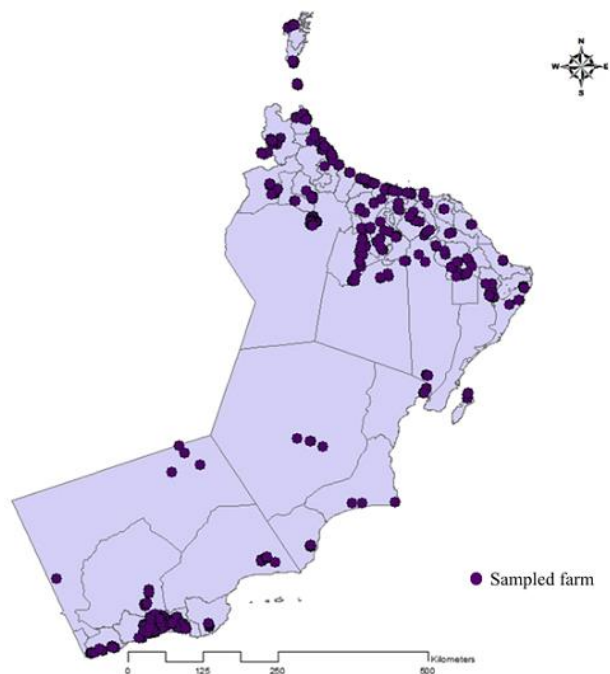Serological screening of ruminants allows CCHF affected

Figure 1. Livestock farms sampled for the mapping of CCHFV in Oman during 2013.

areas to be identified, as antibody prevalence is a good indicator of virus circulation. Virus neutralization assays, generally considered to be highly specific, are rarely used for CCHF diagnosis. Members of the Nairovirus generally induce a weaker neutralizing antibody response (Burt et al., 1993; Rodriguez et al., 1997).

Currently, there is no commercial ELISA available for livestock (OIE, 2014). Few in-house ELISA have been published. Kits are used to replace the conjugate in kit with one that is suitable for animal species to be screened for CCHF specific antibodies (Williams et al., 2000; Adam et al., 2013). Most of the ELISA tests described for livestock have not undergone a formal validation process (Mertens et al., 2013). One of the biggest challenges for such validation is the availability of an adequate number of positive well characterized control samples. In this study, serum samples were collected from animals from which ticks were positive to CCHFV antigen (by ELISA and RT-PCR), and further screened by two types (modified and competitive) ELISA tests to evaluated the indirect modified ELISA due to the presence of inadequate amount of positive control sera supplied with the kit. It has been found that there was agreement between the two ELISA tests.

Keeping in view the public health related importance of disease, this study was aims to estimate the prevalence, and to build a disease distribution of CCHF in livestock in Oman in order to identify areas under high risk and contemplate a comprehensive control strategy. Overall,

prevalence in this study, CCHF antibodies was recorded as 7.1% in livestock in Oman. Many studies conducted elsewhere in the world indicated that cattle are frequently infected with CCHFV, and various prevalence level were observed in Sudan (7.0%) (Adam et al., 2013). CCHF antibodies were detected in 5.9% of sampled cattle in Iran (Lotfollahzadeh et al., 2011). Differences were observed for the sero-prevalence in various livestock species in many studies (Mariner et al., 1995; Hassanein et al., 1997; Mohamed et al., 2008; Telmadarraiy et al., 2010; Albayrak et al., 2012; Adam et al., 2013).

Similarly, 57% of cattle and 31.6% of camels' sera were found positive for antibodies for CCHFV in a study in Niger (Mariner et al., 1995). Sero-prevalence was recorded in Oman as 22% of the livestock, and the highest value recorded as 27% in goat followed by 23 and 3% in sheep and cattle respectively (Williams et al., 2000). This difference could be due to the specificity of the ELISA test used. Tick management practices and farming conditions might affect the higher or lower prevalence in different livestock species kept under the same condition.

Moreover, in this study, animals' sera as well as ticks were found positive for the CCHF indicating the circulation of virus in both hosts. Many authors have documented the antigen detection in ticks collected in various regions of the world through similar methods (Telmadarraiy et al., 2010; Albayrak et al., 2012; Estrada-pena et al., 2012).
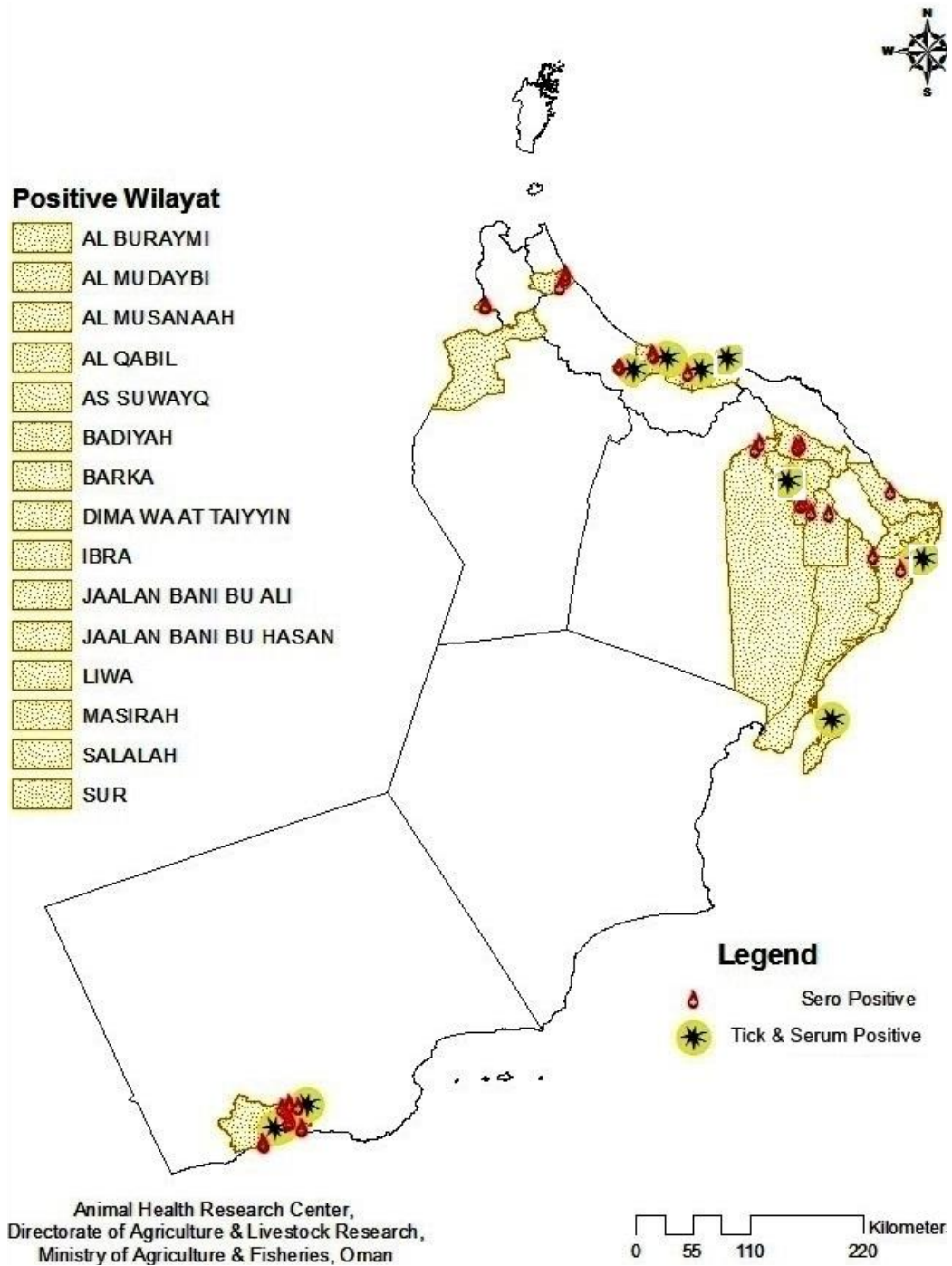
Figure 2. Wilayats and farms where ticks from animals or serum were found positive for CCHFV in Sultanate of Oman.

Conclusion

This study is useful in understanding the dynamic transmission of the virus, and highlighting the regions of high risk in the Sultanate of Oman. Later on, the control measures can be contemplated.

ACKNOWLEDGMENTS
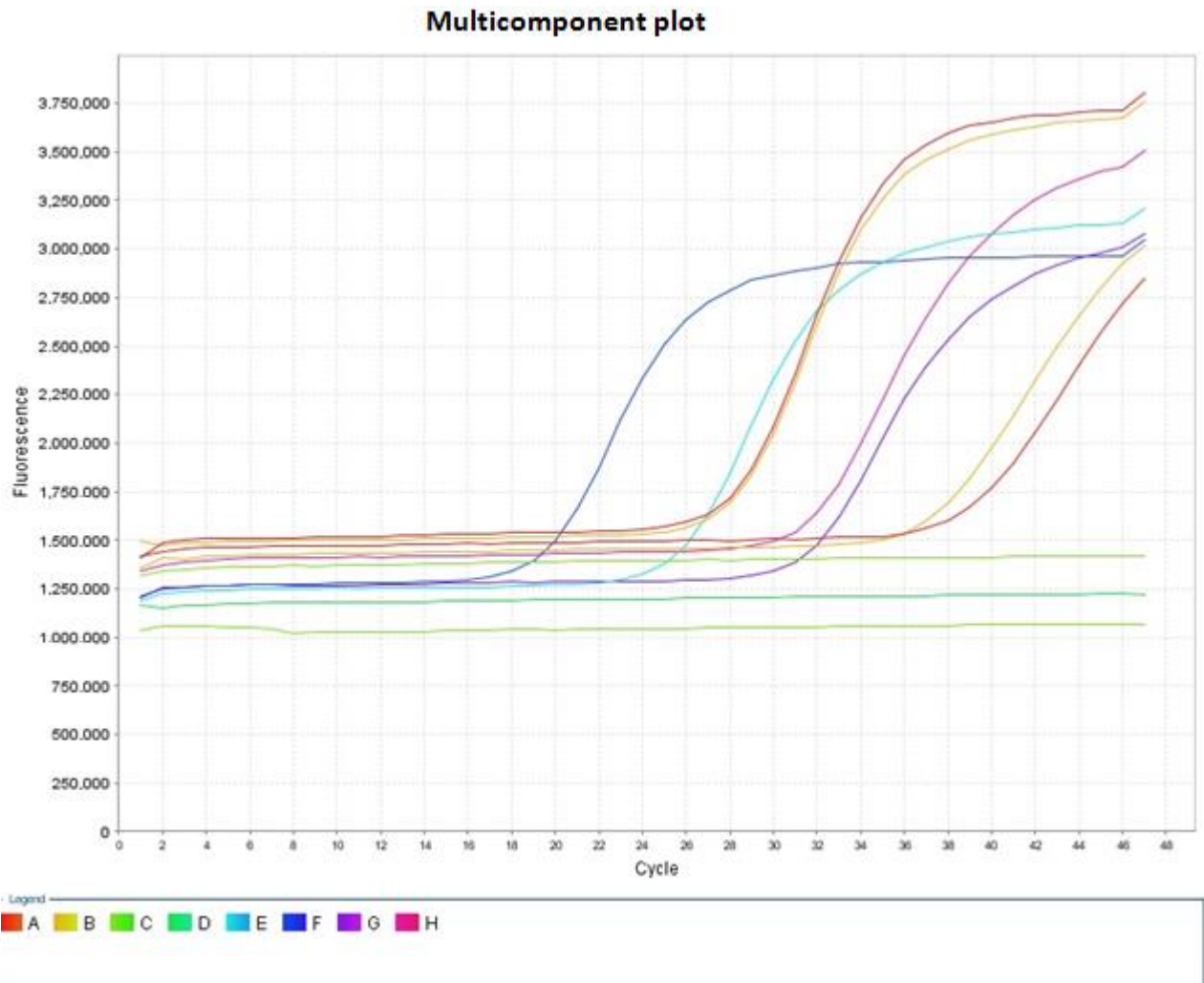
Multicomponent plot

Figure 3. Amplification curves of RNAs extracted from CCHF virus using the RT-PCR (A: control positive, C: control negative, D: negative (by ELISA), B, E, F, G, and H: positive (by ELISA)).

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Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Adam IA, Mahmoud MA, Aradaib IE (2013). A sero-epidemiological survey of Crimean Congo hemorrhagic fever among cattle in north Kordufan state, Sudan .Virol. J. 10:178.

Albayrak H, Ozan E, Kurt M (2012). Sero-survey and molecular detection of Crimean Congo hemorrhagic fever virus (CCHFV) in northern Turkey. Trop. Anim. Health Prod. 44:1667-1671.

Appannanavar SB, Mishra B (2011). An update on Crimean Congo hemorrhagic Fever. J..Glob.Infect. Dis. 3:285-292.

Burt FJ, Swanepoel R, Braack LO (1993). Enzyme-Linked immunosorbent assays for the detection of antibody to Crimean Congo haemorrhagic fever virus in the sera of livestock and wild

vertebrates. Epidemiol. Infect. 111:547-557.

Ergunay K, Whitehouse CA, Ozkul A (2010). Current status of human arboviral disease in Turkey. Vector Borne Zoonotic Dis. 11:7731-7741.

Estrada-pena A, Habela MA, Sanchez N, Palomar AM, Oteo JA, Romero L, Portillo A, Santibanez P (2012). Crimean Congo hemorrhagic fever virus-tick survey in endemic areas in Bulgaria. J. Med. Virol. 84:608-614.

Hassanein KM, EL-Azazy OM, Yousef HM (1997). Detection of Crimean Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudi Arabia. Trans. R. Soc. Trop. Med. Hyg. 91:536-537.

Kamboj A, Pathak H (2013). Crimean Congo hemorrhagic fever; a comprehensive review. Vet.World. 6:812-817.

Lotfollahzadeh S, Nikbakht Boroujeni GR, Mokhber Dezfouli MR, Bokaei S (2011). A Sero-survey of Crimean Congo hemorrhagic fever in dairy cattle in Iran. Zoonoses public Health. 58:54-59.

Maltezou HC, Papa A (2010). Crimean-Congo hemorrhagic fever :risk for emergence of new endemic foci in Europe? Travel Med. Infect. Dis. 8:139-143.

Mariner JC, Morrill J, Ksiazek TG (1995). Antibodies to hemorrhagic fever viruses in domestic livestock in Niger, Rift Vally fever Crimean-Congo hemorrhagic fever. Am. J. Trop. Med. Hyg. 53:217-221.

Mertens M, Schmidt K, Groschup MH (2013). The impact of Cimean Congo hemorrhagic fever virus on public health. Antiviral Res. 98(2):248-260.

Mohamed M, Said AR, Graham R (2008). A serological survey of Crimean Congo hemorrhagic fever in the Sharkia Governorate of Egypt. Vet. Italinna. 44:512-517.

OIE (2014). Crimean Congo Hemorrhagic Fever. Chapter 2.1.3B. pp. 1-8.

Rodriguez LL, Maupin GO, Ksiazek TG (1997). Molecular investigation of a multisource outbreak of Crimean Congo hemorrhagic fever in the United Arab Emirates. Am. J. Trop. Med. Hyg. 57:512-518.

Scrimgeour E (1996). Crimean Congo hemorrhagic fever in Oman. Lancet. 347:692.

Telmadarraiy Z, Ghiasi SM, Moradi M, Vatandoost H, Eshraghian MR, Faghihi F, Zarei Z, Haeri A, Chinikar S (2010). A survey of Crimean Congo hemorrhagic fever in Ardabil province, Iran during 2004-2005. Scand. J. Infect. Dis. 42:137-141.

Watts DM, Ksiazek TG, Linthicum KJ, Hoogstraal H (1989). Crimean Congo hemorrahagic Fever. Vol. 11. In: The Arboviruses: Epidemiology and Ecology (ed.TP Monath). CRC press. Boca Raton. pp. 177-222.

Williams RJ, Al-Busaidy S, Mehta FR, Maupin GO, Wagoner KD, Al-Awaidy S, Suleiman AJ, Khan AS, Peters CJ, Ksiazek TG (2000). Crimean Congo hemorrhagic fever: a sero-epidemiological and tick survey in Sultanate of Oman. Trop. Med. Int. Health 5:99-106.