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Short Communication

Crimean-Congo haemorrhagic fever virus in *Hyalomma impeltatum* ticks from North Kordofan, the Sudan

Lidia Chitimia-Dobler[a,*], Makarim Habeeb Issa[b], Malaz Esamalden Ezalden[c], Idris Ahmed Yagoub[b], Mohamed Abdalsalam Abdalla[d], Amel Omer Bakhiet[d], Sabine Schaper[a], Ramona Rieß[a], Patrick Vollmar[a], Anne Grumbach[a], Malena Bestehorn[e], Markus Antwerpen[a], Gerhard Dobler[a,e], Yassir Adam Shuaib[d]

[a] *Bundeswehr Institute of Microbiology, Munich, Germany*
[b] *Veterinary Research Institute, Soba, Khartoum, Sudan*
[c] *Ministry of Animal Resources, Kassala, Sudan*
[d] *College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum North, Sudan*
[e] *University of Hohenheim, Stuttgart, Germany*

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ABSTRACT

An evidence for Crimean-Congo hemorrhagic fever virus (CCHFV) was found in *Hyalomma impeltatum* ticks collected from sheep in North Kordofan in the Sudan. Based on sequencing of the partial segment S, the detected virus belongs to lineage I with closest similarity to CCHFV strains from Senegal. So far, this lineage is unknown in the Sudan.

Crimean-Congo hemorrhagic fever virus (CCHFV) is a member of the genus *Orthonairovirus* that belongs to the family *Nairoviridae* (Hoogstraal, 1979; Swanepoel, 1994; Whitehouse, 2004; Ergonul, 2006; Schmaljohn and Nichol, 2007). It causes a severe form of hemorrhagic fever known as Crimean-Congo hemorrhagic fever (CCHF) and occurs in a natural enzootic cycle between ticks and vertebrates in some parts of the world (Hoogstraal, 1979; Swanepoel, 1994; Whitehouse, 2004; Ergonul, 2006; Schmaljohn and Nichol, 2007). The geographical distribution of CCHFV largely reflects that of its ixodid tick vectors, particularly those of the genus *Hyalomma* (Hoogstraal, 1979). The tripartite RNA genome of CCHFV consists of small (S), medium, and large segments (Hoogstraal, 1979; Swanepoel, 1994; Whitehouse, 2004; Ergonul, 2006; Schmaljohn and Nichol, 2007). In some endemic regions of Africa, CCHFV is responsible for annual outbreaks with high case-fatality rates of up to 30% (Swanepoel, 1994; Whitehouse, 2004; Ergonul, 2006; Schmaljohn and Nichol, 2007; Wölfel et al., 2009). Outbreaks often affect persons in rural communities such as shepherds and slaughterhouse workers or medical staff at resource-poor hospitals and veterinarians (Ergonul, 2006; Schmaljohn and Nichol, 2007; Wölfel et al., 2009; Aradaib et al., 2010). Despite reported outbreaks and sporadic cases of CCHF in humans in the greater Kordofan area, so far the virus has not been detected and genetically characterized in its presumed tick vectors in the Sudan (Aradaib et al., 2010; Elata et al., 2011). From a public health perspective, confirming CCHFV in ticks will provide a more detailed understanding of its epidemiology as well as will help in identification of high risk areas in the Sudan. We therefore analyzed ticks collected from animals in two different regions in the Sudan to disclose whether these ticks carry CCHFV using molecular characterization tools.

Ticks were collected from domestic animals from North Kordofan and Kassala (Figure 1a) from January to August 2017. A

* Corresponding author at: Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, 80937, Munich, Germany.
E-mail addresses: lydiachitimia@gmail.com (L. Chitimia-Dobler), vet.aboamar@hotmail.com (M.H. Issa), vet.yassir@yahoo.co.uk (M.E. Ezalden), yagoubidris@yahoo.com (I.A. Yagoub), salamaa0002@gmail.com (M.A. Abdalla), amel33@gmail.com (A.O. Bakhiet), SabineSchaper@bundeswehr.org (S. Schaper), ramonariess@bundeswehr.org (R. Rieß), patrickvollmar@bundeswehr.org (P. Vollmar), annegrumbach@bundeswehr.org (A. Grumbach), malena1bestehorn@bundeswehr.org (M. Bestehorn), markusantwerpen@bundeswehr.org (M. Antwerpen), gerharddobler@bundeswehr.org (G. Dobler), vet.aboamar@gmail.com (Y.A. Shuaib).

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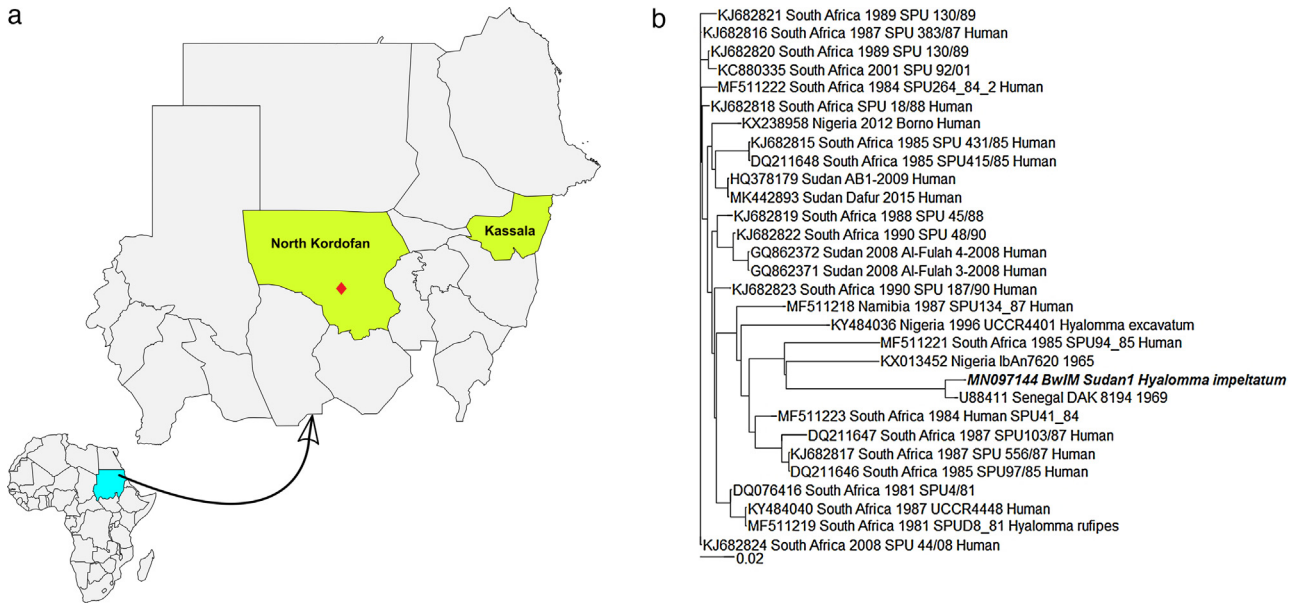

Figure 1. (a) Map of study area showing the sampling location of the positive pool. (b) Maximum-Likelihood tree involving 30 nucleotide sequences of the partial segment S. There were a total of 287 nucleotide positions in the final dataset. Bold: Sequenced isolate from *H. impeltatum* of this study.

total of 2,410 ticks belonging to three genera were morphologically identified according to Apanaskevich and Horak (2009). The *Hyalomma* genus was represented with six species with a total of 998 ticks. The most abundant species was *Hyalomma impeltatum* ticks (60.5%, 598/998). Female *H. impeltatum* ticks were differentiated from other *Hyalomma* females by the vestibular portion or preatrial fold of genital operculum bulging (Apanaskevich and Horak, 2009).

Total nucleic acid was extracted from individual or 2 to 10 pooled ticks (same stage and species and collected from the same host) using the MagNA Pure LC RNA/DNA Kit and instrument (Roche, Mannheim, Germany) according to manufacturer's instructions. Extracted nucleic acid from each individual or pool ticks was tested for CCHFV using qPCR as previously described by Atkinson et al. (2012). One *H. impeltatum* female pool of 10 ticks collected from a sheep in North Kordofan was found to be positive for CCHFV. Moreover, a low density macroarray was applied to confirm the positive screening reaction (Wölfel et al., 2009). A PCR amplifying a 287 bp long fragment of the segment S gave a positive result. Subsequently, the amplicon of the segment S was sequenced by Next Generation Sequencing using Illumina MiSeq® Technology and a ligation-based approach with NEBNext Ultra II DNA (New England Biolabs, Frankfurt, Germany) to study the phylogenetic relationship of the detected strain to previously reported strain sequences available in the NCBI GenBank, including sequences of the strains detected in human cases in Kordofan in 2008.

The minimal infection rate (MIR) was 0.17% (1/598) among *H. impeltatum* ticks and 0.10% (1/998) among the six different *Hyalomma* species. Moreover, the MIR was 0.5% (1/197) from the total number of ticks which were collected from this single host. This low MIR probably indicates that the host was not viremic, therefore, presumably only one of the 10 female *H. impeltatum* in the pool was carrying CCHFV. Based on the engorgement changings, the female ticks of the positive pool had apparently been feeding on the host for two days. The phylogenetic analysis showed that the detected virus belongs to lineage I (GenBank accession number MN097144) and is closely related to CCHFV strains from Senegal (accession number: U88411.1 Senegal DAK 8194 1969) (Figure 1b). This finding is important and it may represent an emerging concern since the formerly reported CCHFV strains in the Sudan belong to lineage 3 (Aradaib et al., 2010; Elata et al., 2011).

Herein, we report for the first time the detection of a strain of CCHFV in ticks in the Sudan. It is phylogenetically different from previously found strains. This finding is significant from a medical point of view since the virus was detected in *H. impeltatum* ticks collected in North Kordofan.

Conflict of interest statement

None of the authors have any conflict of interest (financial or personal) in this study.

Ethical approval

None.

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