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Crimean Congo Hemorrhagic Fever Virus and Alkhurma (Alkhumra) Virus in Ticks in Djibouti

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Abstract

Crimean Congo hemorrhagic fever virus and Alkhurma virus, not previously reported in Djibouti, were detected among 141 (infection rate = 15.7 per 100, 95% CI: 13.4–18.1) tick pools from 81 (37%) cattle and 2 (infection rate = 0.2 per 100, 95% CI: 0.0–0.7) tick pools from 2 (1%) cattle, respectively, collected at an abattoir in 2010 and 2011.

Keywords

Bunyaviridae; Crimean Congo hemorrhagic fever virus; *Flaviviridae*; tick(s)

Crimean Congo hemorrhagic fever virus (CCHFV) and Alkhurma (or Alkhumra) virus (ALKV) are tick-borne pathogens that can cause hemorrhagic febrile illness in infected individuals. CCHFV, of the genus *Nairovirus* and family *Bunyaviridae*, is transmitted by hard ticks of the family Ixodidae. CCHFV is endemic to Africa, the Balkans, the Middle East, and parts of Asia (Bente et al. 2013). ALKV, of the genus *Flavivirus* and family *Flaviviridae*, has been identified in both soft *Ornithodoros savignyi* ticks and hard *Hyalomma dromedarii* ticks (Mahdi et al. 2011). The geographic range of ALKV is less well understood, given the virus' more recent emergence. ALKV has been described primarily in

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Saudi Arabia, although four Italian tourists who visited a camel market on the Egyptian–Sudanese border were infected between 2010 and 2013 (Musso et al. 2015).

To examine the risk of CCHFV and ALKV infection in Djibouti, we collected tick samples over a 20-week period from September 2010 through February 2011 at the Abattoir Frigorifique de Djibouti. The abattoir slaughters livestock imported from Ethiopia and Somalia. At each of the six collections, entomologists from the Ministry of Health of Djibouti (MOH) and U.S. Naval Medical Research Unit No. 3 (NAMRU-3) inspected a convenience sample of freshly slaughtered cattle and removed ectoparasites using blunt forceps. Ticks were stored in cryovials at -70°C at MOH and transferred on dry ice to NAMRU-3 in Cairo, Egypt.

After taxonomic identification, ticks were grouped into pools by species, sex, and source animal. Ticks were homogenized by Mini-Beadbeater-96 (BioSpec, Bartlesville, OK) using specific beads as described (Crowder et al. 2010). RNA was extracted using the QIAamp Viral RNA Kit (QIAGEN, Valencia, CA) according to the manufacturer's instruction. Real-time reverse transcription polymerase chain reaction (PCR) was performed following procedures described elsewhere for detection of the CCHFV S segment (Garrison et al. 2007) and ALKV (Carletti et al. 2010). Positive controls were used to evaluate results, and positive pools were confirmed by sequence (Garrison et al. 2007; Carletti et al. 2010). ALKV RNA from Saudi Arabia was provided by Viral Special Pathogens Branch (U.S. Centers for Disease Control and Prevention, Atlanta, Georgia) for use as a positive control.

Infection rates were calculated using maximum likelihood estimates and skewness-corrected score confidence intervals (Biggerstaff 2009). Infection rates are reported per 100 ticks.

A total of 953 ticks were collected, with an average of 190 (range 118–303) ticks collected from an average of 44 (range 30–73) animals at each sampling. One hundred seventy-one (78%) cattle from which ticks were collected were imported from Ethiopia and one (<1%) from Somalia; country of origin is unknown for 48 (22%) cattle. All ticks were of the family Ixodidae, with 518 (54%) belonging to the genus *Amblyomma*, 316 (33%) to *Hyalomma*, 96 (10%) to *Dermacentor*, and 23 (3%) to *Rhipicephalus*.

Of the 546 pools into which ticks were grouped, 141 (15.7, 95% CI: 13.4–18.1) pools from 81 (37%) cattle were positive for CCHFV and 2 (0.2, 95% CI: 0.0–0.7) pools from 2 (1%) cattle were positive for ALKV (Table 1). Sixty-two (77%) cattle from which CCHFV-positive ticks were collected and two (100%) cattle from which ALKV-positive ticks were collected originated in Ethiopia. Country of origin is unknown for the remaining cattle from which CCHFV-positive ticks were collected. No tick pools were infected with both pathogens.

To our knowledge, this is the first evidence of either CCHFV or ALKV in Djibouti. However, it is not possible to determine whether PCR-positive ticks fed on infected animals within Djibouti or along the route by which cattle were imported from neighboring countries. Despite this uncertainty, the identification of both viruses in Djibouti adds to understanding of tick-borne pathogens in the country, which is currently limited.

Djibouti falls within the known endemic region for CCHFV. The virus has been identified in nearby Sudan and Kenya (Bente et al. 2013), and analyses of CCHFV strains in the Arabian Peninsula suggest that livestock from the Horn of Africa may be responsible for outbreaks there (Deyde et al. 2006). Serologic evidence of CCHFV infection has been found among an abattoir worker population in Djibouti (Horton et al., unpublished data). Presence of CCHFV in ticks in the country adds further evidence to suggest that CCHFV may circulate within Djibouti.

ALKV has not been reported previously in the Horn of Africa, so results from this study suggest that the geographic range of ALKV may be larger than previously described, regardless of whether PCR-positive ticks fed on infected animals in Djibouti or elsewhere along the route of importation from Ethiopia.

CCHFV infection rates were highest in *Rhipicephalus spp.* (27.6, 95% CI: 12.0–48.9), although the sample size in this genus was small so there is substantial uncertainty in this estimate. The next highest infection rate was found in *Hyalomma spp.* (21.2, 95% CI: 16.9–26.1), followed by *Amblyomma spp.* (12.4, 95% CI: 9.8–15.5) and *Dermacentor spp.* (12.1, 95% CI: 6.6–20.1). *Hyalomma spp.* is the principal vector of CCHFV, but there is little evidence that the other genera in which CCHFV was identified in this study have a role in maintenance or transmission (Papa et al. 2004), so positive ticks likely fed on infected animals.

ALKV was detected in *Amblyomma lepidum*, a tick found in East Africa and Iran (Piazak 2005), in which ALKV has not been previously identified. However, the vector competence of this species is not known, and positive results from engorged ticks may reflect viremic host blood in the ticks. It is notable that none of the *Hyalomma dromedarii* ticks tested positive for ALKV.

As the incidence of tick-borne disease increases globally, surveillance among tick populations is a potential strategy to improve understanding of virus circulation and risks to human health. Given the evidence of CCHFV and ALKV among ticks in this study, clinicians and public health officials in Djibouti should be aware of the possibility of these infections in human populations. However, further research and surveillance will be important to fully evaluate CCHFV and ALKV risks in this region.

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Table 1
Number of CCHFV- and ALKV-Positive Tick Pools by Tick Species with Infection Rates per 100 Ticks and 95% Confidence Intervals

Tick species	CCHFV			ALKV			TOTAL	
	n	Infection rate (95% CI)	n	Infection rate (95% CI)	Tick pools	Total ticks		
<i>Amblyomma</i>	61	12.4 (9.8–15.5)	2	0.4 (0.1–1.3)	266	518		
<i>cohaerens</i>	9	18.1 (9.4–30.4)	0	0.0 (0.0–6.8)	35	51		
<i>detritum</i>	0	0.0 (0.0–39.2)	0	0.0 (0.0–39.2)	2	4		
<i>lepidum</i>	41	12.3 (9.1–16.1)	2	0.6 (0.1–1.8)	168	356		
<i>variegatum</i>	11	10.5 (5.7–17.3)	0	0.0 (0.0–3.4)	61	107		
<i>Dermacentor</i>	11	12.1 (6.6–20.1)	0	0.0 (0.0–3.8)	50	96		
spp.	11	12.1 (6.6–20.1)	0	0.0 (0.0–3.8)	50	96		
<i>Hyalomma</i>	63	21.2 (16.9–26.1)	0	0.0 (0.0–1.2)	209	316		
<i>dromedarii</i>	3	16.4 (4.7–37.5)	0	0.0 (0.0–16.9)	14	18		
<i>excavatum</i>	1	9.6 (0.6–37.6)	0	0.0 (0.0–25.4)	7	10		
<i>impehlatum</i>	0	0.0 (0.0–79.4)	0	0.0 (0.0–79.4)	1	1		
<i>magnatum</i>	59	22.4 (17.8–27.7)	0	0.0 (0.0–1.3)	182	282		
<i>marmoreum</i>	0	0.0 (0.0–79.4)	0	0.0 (0.0–79.4)	1	1		
<i>truncatum</i>	0	0.0 (0.0–56.2)	0	0.0 (0.0–56.2)	3	3		
<i>turanicum</i>	0	0.0 (0.0–79.4)	0	0.0 (0.0–79.4)	1	1		
<i>Rhipicephalus</i>	6	27.4 (12.0–48.9)	0	0.0 (0.0–14.2)	21	23		
<i>annulatus</i>	2	30.6 (5.9–71.0)	0	0.0 (0.0–34.1)	6	7		
<i>decoloratus</i>	0	0.0 (0.0–79.4)	0	0.0 (0.0–79.4)	1	1		
spp.	4	27.6 (9.6–54.2)	0	0.00 (0.0–20.2)	14	15		
Total	141	15.7 (13.4–18.1)	2	0.2 (0.0–0.7)	546	953		

ALKV, alkhumra virus; CCHFV, Crimean Congo hemorrhagic fever virus.