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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...
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.

**Acknowledgments**

We would like to thank the management and workers from the abattoir for their cooperation and support during specimen collection. We also recognize members of the MOH Vector Biology Unit, led by Mr. Abro Moussa and Mr. Houssein Omar, and Ms. Rania Kaldas and Mrs. El Shaimaa Nour Eldin of the NAMRU-3 Vector Biology Research Program who conducted specimen collection and identification.

**References**

Biggerstaff, BJ. PooledInfRate, Version 4.0: A Microsoft® Office Excel© Add-In to Compute Prevalence Estimates from Pooled Samples. Fort Collins, CO: Centers for Disease Control and Prevention; 2009.


<table>
<thead>
<tr>
<th>Tick species</th>
<th>CCHFV</th>
<th>ALKV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyomma cohærens</td>
<td>61</td>
<td>12.4 (9.8–15.5)</td>
</tr>
<tr>
<td>Detritum</td>
<td>0</td>
<td>0.0 (0.0–39.2)</td>
</tr>
<tr>
<td>Ixodes lepidum</td>
<td>41</td>
<td>12.3 (9.1–16.1)</td>
</tr>
<tr>
<td>Variegatum</td>
<td>11</td>
<td>10.5 (5.7–17.3)</td>
</tr>
<tr>
<td>Dermacentor variabilis</td>
<td>11</td>
<td>12.1 (6.6–20.1)</td>
</tr>
<tr>
<td>Hyalomma dromedarii</td>
<td>3</td>
<td>16.4 (4.7–37.5)</td>
</tr>
<tr>
<td>Hyalomma excavatum</td>
<td>1</td>
<td>9.6 (0.6–37.6)</td>
</tr>
<tr>
<td>Hyalomma impeltatum</td>
<td>0</td>
<td>0.0 (0.0–79.4)</td>
</tr>
<tr>
<td>Hyalomma marginatum</td>
<td>59</td>
<td>22.4 (17.8–27.7)</td>
</tr>
<tr>
<td>Hyalomma marmoreum</td>
<td>0</td>
<td>0.0 (0.0–79.4)</td>
</tr>
<tr>
<td>Rhipicephalus truncatus</td>
<td>0</td>
<td>0.0 (0.0–56.2)</td>
</tr>
<tr>
<td>Rhipicephalus turanicum</td>
<td>0</td>
<td>0.0 (0.0–79.4)</td>
</tr>
<tr>
<td>Rhipicephalus annulatus</td>
<td>6</td>
<td>27.4 (12.0–48.9)</td>
</tr>
<tr>
<td>Rhipicephalus decoloratus</td>
<td>2</td>
<td>30.6 (5.9–71.0)</td>
</tr>
<tr>
<td>Rhipicephalus spp.</td>
<td>0</td>
<td>0.0 (0.0–79.4)</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>15.7 (13.4–18.1)</td>
</tr>
</tbody>
</table>

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