Transmission of Crimean-Congo haemorrhagic fever virus from experimentally infected sheep to *Hyalomma truncatum* ticks

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SUMMARY

Crimean-Congo haemorrhagic fever (CCHF) virus was inoculated into West African sheep that were simultaneously infested with adult *Hyalomma truncatum* ticks. Certain sheep developed a viraemia and antibodies, indicating virus infection and replication; however, the length and magnitude of the viraemia and serological responses corresponded to the animals' immunological status. Tick attachment and feeding was not influenced by sheep infection. CCHF virus infection was acquired by 11-33% of female and 0-60% of male ticks. Infection in the ticks did not influence their feeding success, as judged by weight at drop-off, and the weight of eggs produced by infected and non-infected ticks was similar. Transovarial transmission of CCHF virus was demonstrated in 2 of 12 (17%) egg batches from infected female ticks, but in none of 19 egg batches from ticks that tested negative for CCHF virus. Our results suggest that under certain ecological conditions, sheep may serve to amplify CCHF virus in nature through horizontal transmission and that the maintenance cycle also may be influenced by transovarial transmission to the next generation of ticks.


INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) virus (family *Bunyaviridae*, genus *Nairovirus*) is a virus transmitted enzootically by ixodid ticks that may cause severe disease in humans (Hoogstraal, 1979). Remarkably widespread in its geographic distribution, CCHF virus is found throughout much of the southern Soviet Union, southern Europe, central Asia, the Middle East.
and Africa. It occurs in 3 of the world’s 7 major biotic zones (Watts et al., 1988). Equally astonishing is the diversity of potential tick vectors and vertebrate reservoirs; at least 31 species from 9 genera of Ixodid and Argasid ticks have been found to harbour CCHF virus (Camiaas et al., 1990), and more than 20 different vertebrate species have been found to be naturally infected with the virus (Watts et al., 1988). Human disease, which is often severe and sometimes fatal (e.g. Swanepeel et al., 1990), has been recognized from more than a dozen countries on 3 continents (Watts et al., 1988).

Despite an abundance of research documenting the widespread distribution of CCHF virus, its possible vectors and potential vertebrate reservoirs, our understanding of the transmission cycle remains incomplete.

Factors that influence zooneic transmission of CCHF virus include the density of competent vector ticks and the relative abundance of vertebrates that serve as tick hosts or potential vertebrate reservoirs (Hoogstraal, 1979). Sustained transmission is found only where species of Hylaommia ticks are present, and epizootic or epidemic transmission is believed to occur during periods of increased abundance of these ticks.

We recently documented such epizootic transmission in northern Senegal that corresponded temporally with a sudden increase in the abundance of adult H. truncatum and H. impeltatum (Wilson et al., 1990a). Similarly, we have demonstrated a spatial correlation between the relative abundance of H. hyalomma ticks and the prevalence of IgG antibodies against CCHF virus there (Wilson et al., 1990b). Finally, the feeding patterns, host preferences and ecological associations of Ixodid ticks in West Africa (Mored, 1969) suggest that Hylaommia spp. are most likely to serve as enzootic vectors (Camiaas et al., 1990).

Vegetrate hosts for most of the Ixodid ticks considered to be likely vectors include small mammals or birds for the larval and nymphal stages, and large mammals for adult ticks. Domestic ungulates are the principal hosts of most adult Hylaommia ticks, and the prevalence of antibodies against CCHF virus in these animals indicates that they also are frequently infected (Watts et al., 1988; Gonzalez et al., 1990; Wilson et al., 1990a, b). Because sheep are among the most common tick-infested animals in many regions where CCHF virus circulates, they may serve a role in horizontal transmission of the virus.

Accordingly, we studied whether adult ticks feeding on sheep viremic with CCHF virus may be infected, and whether that infection may be transmitted transovarially to the next generation of ticks.

### MATERIALS AND METHODS

#### Virus

The strain of CCHF virus (Dak H49199) used in these experiments was originally isolated in 1988 from a fatal human case in Rosso, Mauritania (Gonzalez et al., 1990). Following 3 consecutive intracerebral mouse passages, the titre was log 6.5 LD50/ml. Virus identification was performed on ground mouse brains using a previously described antigen capture (Saluzzo and Legganno, 1987). Confirmation of the identity of virus was made by the CF test at the World Health Organization Reference Center for Arboviruses at the Pasteur Institute in Dakar.

#### Infection and transmission

Two days prior to virus inoculation, each sheep was infected with 30 female H. truncatum. An elastic cloth stockinette was wrapped around the neck, and the end was glued to the base of the shaved tails of each sheep. The ticks were placed in the bag which was then sealed and then were allowed to attach. Sheep were inoculated ip with 10^5.5 LD50 of CCHF virus. All sheep were bled daily by venipuncture, their temperature was taken and their general condition noted. Beginning 4 days p.i., the tail bags were examined daily; engorged female ticks that had naturally finished feeding were removed. Male ticks, which remained attached to sheep for a longer time, were detached by forceps at 16 days p.i.

Fed ticks were weighed, and individual engorged females were held at 25°C and 75% RH in 3.5 g acrylimaldehyde plastic vials closed with a fine mesh cap until egg laying ended. Tick eggs were suspended in water ground individually in Hanks’ solution, centrifuged and inoculated into NB mice or cell culture. Tick eggs were treated with approximately of about 100 eggs per batch were ground and inoculated into cell culture.

### CCHF VIRUS INFECTION OF HYALOMMA TRUNCATUM BY SHEEP

Serology assays

### Virus replication assays

Virus replication was attempted by inc. inoculation of NB mice and/or inoculation of Vero cells, which produced 10 and 10-fold-diluted sera. Virus identification in cell culture was made by indirect immunofluorescent antibody test on Vero cells, using polyclonal and monoclonal antibodies.

An antigen capture ELISA (saluzzo and Legganno, 1987) was also employed to test for the presence of CCHF viral antigens in NB mouse brain and cell culture supernatant. Plates were coated first with anti-human p-chain-specific IgM antibody, followed by human sera with high-titre IgM. The test sera were added next and then a hightitre anti-CCHF virus monoclonal IgG was added to bind to any antigen captured from the serum. An anti-human IgG, conjugated with horseradish peroxidase, and the chromatogenic substrate, were used for colorimetry.
RESULTS

Sheep serology and viraemia

Inoculated sheep exhibited high-titre antibodies to CCHF virus, although the temporal pattern differed with their past exposure to the virus, as shown in Table 1. The previously exposed sheep (no. 1) produced a slightly elevated IgM titre (1/100) and maintained high IgG (1/10,000). Its offspring (no. 2) showed weakly elevated IgM (1/100) beginning 9 days p.i. and an increase in IgG that developed slowly over the period of study. The naive sheep (no. 3) developed high-titre IgM (1/1,000) beginning 6 days p.i. followed by elevated IgG. No antibody was detected in the control sheep (no. 4).

Viraemia duration (0-6 days) and magnitude (5 x 10^5 LD_{so}/ml) also corresponded to previous exposure (Table 1). Viraemia was undetectable in the previously exposed sheep, mild and brief in its offspring, elevated and longer-lasting in the naive sheep, and non-existent in the control.

Adult tick feeding and infection

A total of 108 male (90%) and 109 female (91%) ticks attached to the sheep and were removed alive for further study. The rate of attachment per sheep varied from 70-97% for male and 80-100% for female ticks, but no systematic differences in tick feeding among sheep were evident (Table 1). Engorged female ticks began detaching 6 days p.i., with similar drop-off patterns (Fig. 2). All female ticks that attached took a blood-meal although their mean weights at detachment differed among sheep (Table 1). Male tick weights were similar.

Virus was detected in 1 or more ticks that fed on each of the 3 inoculated sheep; however, the percentage of infected ticks differed markedly (Table 1). The previously inoculated sheep (no. 1) infected 11% of the female and none of the male ticks tested. More of the ticks feeding on offspring (no. 2) were infected: 14% of females and 30% of males. The sheep that previously was naive to CCHF virus or antibodies (no. 3) infected 33% of females and 58% of male ticks tested. None of the ticks from the control sheep (no. 4) were infected.

The period of detectable viraemia corresponded to the time during which infected female ticks were detaching (Fig. 2). Ticks which detached after the viraemia had become undetectable, with 1 exception, were not infected. Two of 18 ticks feeding on sheep no. 1, which never produced a detectable viraemia, were nevertheless infected.

The weights of fed ticks varied among the
Table II. Weights of adult *H. truncatum* ticks feeding on CCHF virus-infected and non-infected sheep.

<table>
<thead>
<tr>
<th>Weight (X (+/- SD) mg) of ticks or eggs</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. 1 (ad. female)</td>
<td>no. 2 (juv. female)</td>
</tr>
<tr>
<td>Female ticks</td>
<td></td>
</tr>
<tr>
<td>infected</td>
<td>512.9 (+9.6)</td>
</tr>
<tr>
<td>uninfected</td>
<td>523.3 (+15.8)</td>
</tr>
<tr>
<td>Male ticks</td>
<td></td>
</tr>
<tr>
<td>infected</td>
<td>14.5 (+0.9)</td>
</tr>
<tr>
<td>uninfected</td>
<td>13.5 (+1.5)</td>
</tr>
<tr>
<td>Egg batches</td>
<td></td>
</tr>
<tr>
<td>infected</td>
<td>284.2</td>
</tr>
<tr>
<td>uninfected</td>
<td>153.6 (+54.2)</td>
</tr>
</tbody>
</table>

Sheep, but infected and uninfected tick weights were not significantly different (table II). Male ticks, which weighed 11.3 (+1.0) mg prior to attachment, ranged between 13.6 (+1.5) mg and 15.2 (+0.6) mg upon their removal. Female ticks, whose size and weight increase many-fold during feeding, weighed 13.9 (+0.9) mg prior to feeding and ranged between 512.9 (+9.6) mg and 828.1 (+52.1) mg at drop-off. Curiously, the weight of engorged females declined as the length of time to dropoff increased (fig. 3). No systematic difference between the weight of infected and non-infected ticks was found (table II).

Transovarial transmission

Eggs were laid by 102 of 109 (94%) engorged females (3 engorged ticks died prior to oviposition and 1 was crushed in the tail bag). Individual eggs were not counted, but the weights of eggs laid by infected female ticks did not differ from those of uninfected ticks (table II). CCHF virus antigen was detected in 2 of 12 (17%) egg batches from infected female ticks. Specifically, 1 of 2 and 1 of 3 egg batches from infected ticks of sheep no. 1 and no. 2, respectively, tested positive; all 7 egg batches from infected ticks of sheep no. 3 tested negative. None of 19 egg batches from female ticks testing negative were positive.

**Discussion**

Adult *H. truncatum*, a potential enzootic vector of CCHF virus, became infected while feeding on sheep that were viraemic. Sheep were chosen as the vertebrate host for this experiment because they are heavily infested by numerous tick species in West Africa (Gueye et al., 1977; Umoh et al., 1983) and because serological surveys have demonstrated that they are frequently infected with CCHF virus (Camicas et al., 1990). Nevertheless, the most efficient transmission of CCHF virus to feeding ticks occurred on sheep that had not been infected previously, and hence that developed the most intense and lasting viraemia. Ticks successfully engorged on all sheep, and CCHF virus infection did not appear detrimental to their feeding, survival or reproduction. A high rate of attachment was observed on each sheep, demonstrating that feeding on the tails of these hosts was effective. The timing of female tick drop-off was similar among all sheep in this experiment, and was like that previously observed when *H. truncatum* fed on other uninfected sheep (T.M. Logan and M.L. Wilson, unpublished). Eggs were laid by most of the ticks from each sheep, indicating that mating had occurred. Interestingly, both female ticks, which consumed large quantities of host fluids, and males, whose fluid uptake was small, became infected. Whether some ticks were infected by the genital route was not determined in our study, although controlled observations to answer this question would be useful.

Other experimental efforts to demonstrate transmission of CCHF virus to *H. truncatum* also have suggested that this tick is a competent vector. *Nymphal H. truncatum* that were inocu-
lated intracereally replicated CCHF virus (Shepherd et al., 1989). Similarly, larval *H. truncatum* that fed on infected NB mice replicated and transtadially transmitted CCHF virus (Logan et al., 1989). Virus remained detectable in adult ticks for more than 200 days p.i. in these latter studies, suggesting a possible mechanism for interepidemic maintenance (Shepherd et al., 1989).

Transovarial transmission (TOT) of CCHF virus by female *H. truncatum* ticks to their eggs was observed only twice in our experiment. None of the egg batches from female ticks testing negative were positive, suggesting that the method was specific. Experimental TOT of CCHF virus has been observed previously in *H. marginatum rufipes* (Lee and Kemp, 1970) and *H. m. marginatum* (Kondratenko et al., 1970; Zgurskaya et al., 1971; Levi and Vasilenko, 1972). However, the frequency of TOT of this virus in *Hyalomma* ticks apparently occurs rarely (Shepherd et al., 1989). While some ticks may serve as both vector and reservoir to CCHF virus, the epizootic transmission of CCHF virus (Wilson et al., 1990a, b) was observed among sheep in northern Senegal. The reported transmission of CCHF virus (Wilson et al., 1990a). Also, a spatial correlation between *Hyalomma* tick abundance and CCHF viral antibody in humans and sheep has been demonstrated (Wilson et al., 1990b). Finally, we recently have completed a study demonstrating that being bitten by an adult male *H. truncatum* is a major risk factor for human CCHF virus infection in northern Senegal (Chapman et al., 1991). Results of the present study further support the role of *H. truncatum* in the transmission of CCHF virus, and raise the possibility that sheep may serve as amplifying hosts in the enzootic cycle.

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Transmission experiment of CCHF virus of moultion to a moultion to the tick *Hyalomma truncatum*

Le virus de la fièvre hémorragique de Crimée-Congo (CCHF) a été inoculé à des moultions d’Afrique occidentale simultanément infectées avec des tiques *Hyalomma truncatum* adultes. Certaines moultions ont fait une virémie et produisent un anticorps, signant l’infection et la réplication virale. Toutefois, la durée de l’aptitude de la virémie et de la réponse sérologique correspond à l’éventualité d’un contact préalable avec le virus. La fixation et le repas des tiques n’ont pas influencé par l’infection des moultions. Le virus CCHF est transmis à 11-13 % des tiques femelles et à 50-60 % des tiques mâles. L’infec- tion des tiques n’a pas d’incidence sur leur virémie se gorgent s’il on leur juge par le poids des femelles gorgées et par le fait que le poids de la ponte est semblable entre les deux groupes. La prouve de la trans- mission transovarienne a été obtenue dans 2 pontes sur 12 (17 %) provenant de femelles infectées.

Nos résultats suggèrent que, dans certaines condi- tions écologiques, les moultions pourraient amplifier ce virus dans la nature, par transmission horizontal, et que le cycle d’entretien peut être influencé en partie par la transmission transovarienne à la génération de tiques suivante.

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