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Sexual and transovarian transmission of Crimean-Congo haemorrhagic fever virus in *Hyalomma truncatum* ticks

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SUMMARY

Male *Hyalomma truncatum* ticks were inoculated with Crimean-Congo haemorrhag-

western Africa (Watts *et al.*, 1988). Fundamental knowledge of the mechanisms of replication and transmission is a prerequisite for understanding the virus ecology. We previously reported on CCHF virus replication in adult *Hyalomma truncatum* and *Amblyomma variegatum* ticks (Gonzalez *et al.*, 1991), two major vectors of CCHF virus in West Africa (Camicas *et al.*, 1990); little is known, however, about transmission.

Classically, CCHF virus is acquired by an arthropod during a bloodmeal from a viraemic host. However, many infected hosts apparently exhibit a brief, sometimes undetectable, viraemia (Gonzalez *et al.*, 1989b). Other mechanisms may play a role in increasing the rate of CCHF virus infection of ticks in nature. Transovarial

by the intra-anal route with a volume of 2 µl of the diluted CCHF virus supension (Gonzalez *et al.*, 1989a). They were then placed on a laboratory rabbit for 11 days before hypostomectomy and the introduction of females.

Tick hypostomectomy and genital sealing

Adult males were immobilized on their backs using a cork board and overlapping pins that secured but did not damage the tick. After the palps were spread apart, the hypostome was cut using opthalmic scissors. The chelicera were untouched. Formation of a haemolymph clot was immediate. Specimens were inspected after hypostomectomy and the quality of the section was confirmed by a classical scanning electron microscopy (Kruger and Hamilton-Atwell, 1988) (fig. 1).

For 6 female ticks, their genital aperture was

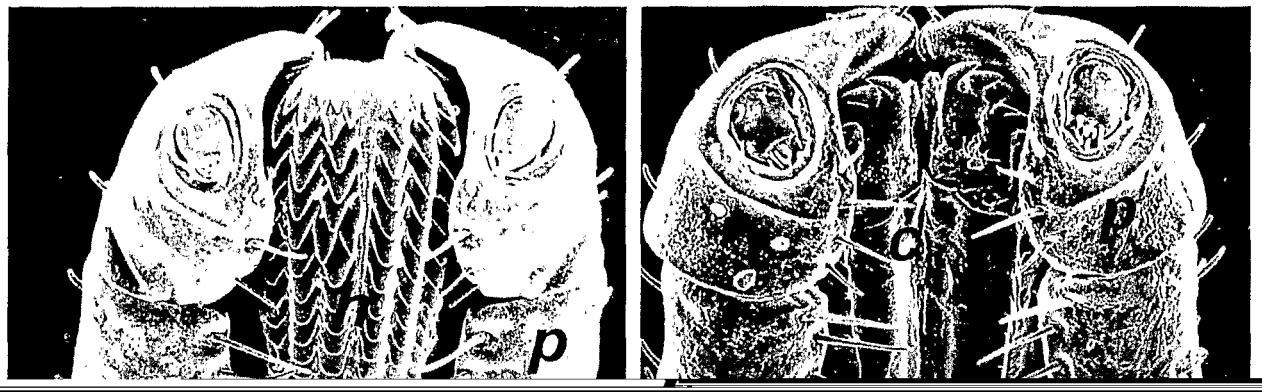
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Table I. Transmission of CCHF virus by mating between infected adult male *H. truncatum* ticks.

Exp. no.	Trans-mission studied	Tick pre-infection	Tick treatment: Hypostom-ectomy (male)	Tick treatment: Gonopore occlusion (female)	Virus isolates (no. tested): Adult Males	Adult Females	F_1 Eggs [a]	F_1 Larvae [a]	F_1 Nymphs [b]	Rabbit seroconversion: Male pre-feeding	Female and male cofeeding
1	Mating	Males	Yes	No	2 (3) [c]	4 (6) 0 (2) [e]	2 (4) [d] 0 (4) [e]	1 (6) [d] None [f]	0 (15) [d] None	+	+
2	Cofeeding	Males	No	Yes	3 (3)	3 (3)	None	None	None	+	+
3	Control	Males	Yes	Yes	3 (3)	0 (3)	None	None	None	+	−
4	Control	Females	Yes	Yes	0 (3)	3 (3)	None	None	None	−	+
5	Control	None	No	Yes	0 (3)	0 (3)	None	None	None	−	−

[a] Each observation was pooled from the eggs of one female.
[b] Nymphs from positive pools of larvae.
[c] A fourth tick that was dead tested negative.
[d] Pools from previous stage that tested positive.
[e] Pools from previous stage that tested negative.
[f] Not produced/not alive.

produced by the 4 virus-positive females (table I). From these positive egg batches, 6 larval pools were tested, and virus was isolated from one of them at a titre of 3.7 log LD_{50}/ml. Attempts to isolate the virus from 15 nymph pools derived from the larvae of the batch that tested positive were negative. The rabbits fed upon by preinfected, hypostomectomized males cofeeding with females also had seroconverted 15 days postinfestation (antibody titre > 1,600), presumably from infection via the female ticks.

In the cofeeding experiment (exp. 2), involving hypostome-intact males and gonopore-closed females, each female tick that was associated with an inoculated male became infected. The rabbit seroconverted, demonstrating virus transmission from the male ticks. Apparently these gonopore-closed female ticks became infected by feeding on the rabbit.

In one control experiment (exp. 3) involving preinfected, hypostomectomized males and gonopore-closed females, no virus was isolated from the engorged females. All male ticks tested positive but the rabbit on which males and females were placed did not seroconvert (table I). In another control (exp. 4), 3 preinfected females, which tested positive after feeding, did not transmit CCHF virus to 3 hypostomectomized males in the same capsule; the rabbit, however, seroconverted. Finally (exp. 5), the virus was not isolated from non-infected, hypostome-intact male ticks, nor from the non-infected gonopore-closed female ticks. The rabbit had not seroconverted when tested 15 days after infestation.

All rabbits that served in the initial "prefeeding" exposure of preinfected male ticks seroconverted following infestation (antibody titre > 800). No rabbits prefed upon by infected males showed evidence of CCHF antibody.

DISCUSSION

By altering the ability of male *H. truncatum* to feed and/or of female ticks to become inseminated, we have demonstrated that CCHF virus can be sexually transmitted to females from experimentally preinfected males. Preinfected females, however, did not sexually transmit CCHF virus to hypostomectomized males. Virus apparently was not transmitted either to female ticks or to the host by leakage of saliva or other infected secretions. From these observations, it appears that the male spermatophore served as the means by which CCHF virus was transmitted to the female. These infected female ticks in turn became infectious for their rabbit hosts.

Moreover, the virus was vertically transmitted from the females through their eggs at least to the first developmental stage.

When sexual transmission was prevented, CCHF virus was passed horizontally from male to female ticks, apparently by cofeeding. Horizontal transmission in ticks may take place by several means. Classically, it occurs when vectors feed during the time that vertebrate hosts are viraemic. However, other mechanisms have been suspected, as host viraemia is often undetectable. CCHF virus transmission has been observed when non-infected ticks cofed with infected ones (Logan *et al.*, 1989). Even when viraemia is undetected, Thogoto virus transmission was observed by "distant" cofeeding on a non-viraemic host (Jones *et al.*, 1987). Another rare mode of transmission could occur during hyperparasitism of the infected females by males (Ntiamoa-Baidu, 1986 *in* Logan *et al.*, 1989). Recently, we demonstrated horizontal transmission of CCHF virus from a weakly immune non-viraemic preimmunized sheep to adult *H. truncatum* (Wilson *et al.*, 1991).

Vertical transmission of CCHF virus has been demonstrated by TOT (Lee and Kemp, 1970). However, rates of infection are low, ranging

ed eggs than those infected by cofeeding or as immature ticks. In such cases, sexual transmission of CCHF virus might be an important means by which CCHF virus is maintained in natural populations of *H. truncatum*.

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Transmission sexuelle expérimentale du virus de la fièvre hémorragique de Crimée-Congo chez la tique *Hyalomma truncatum*

Des mâles de *Hyalomma truncatum* ont été infectés expérimentalement avec le virus de la fièvre hémorragique de Crimée-Congo (CCHF). Après la

que. Leur rôle de vecteur efficace est la résultante de leur aptitude à s'infecter, répliquer et transmettre le virus CCHF. La transmission sexuelle, suivie d'une transmission transovarienne, pourrait participer au maintien du virus dans la nature, en augmentant le taux d'infection du vecteur.

Mots-clés: Bunyaviridae, Virus CCHF, Tique; *Hyalomma truncatum,* Transmission sexuelle et transovarienne, Cofeeding, Afrique.

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