Epidemiol. Infect. (1991), **106**, 373–382
Printed in Great Britain

Viraemic transmission of Crimean-Congo haemorrhagic fever virus to ticks

A. J. SHEPHERD[1]*, R. SWANEPOEL[1]†, S. P. SHEPHERD[1], P. A. LEMAN[1] AND O. MATHEE[2]

[1] *Department of Virology, University of the Witwatersrand and Special Pathogens Unit, National Institute for Virology, Sandringham 2131, Republic of South Africa*
[2] *Tick Research Unit, Veterinary Research Institute, Onderstepoort, Republic of South Africa*

(*Accepted 16 October 1990*)

SUMMARY

In order to determine the way in which vertebrates infected with Crimean-Congo haemorrhagic fever (CCHF) virus and potential ixodid tick vectors interact in nature, immature and adult ticks of several species were fed on viraemic mammals and then assayed for virus content at varying times after feeding. CCHF virus was not isolated from ticks of six species tested after feeding as adults and immature forms on sheep with viraemia of $10^{2.5-3.2}$ LD 50/ml, nor from larval ticks fed on guinea-pigs and white-tailed rats with viraemia of $10^{1.9-2.7}$ LD 50/ml. In contrast, virus was isolated from 10 of 152 pools of engorged adult ticks of 5 species that fed on cattle with viraemia of $10^{1.5-2.7}$ LD 50/ml and from 3 of 137 female ticks after oviposition. Infection was transmitted to larval and nymphal *Hyalomma truncatum* and *H. marginatum rufipes*, but not to *Rhipicephalus evertsi evertsi*, from a scrub hare with viraemia of $10^{4.2}$ LD 50/ml but only nymphal *H. truncatum* and *H. m. rufipes* became infected from scrub hares with viraemia of $10^{2.6-2.7}$ LD 50/ml. Infection was transmitted trans-stadially in *H. m. rufipes* and *H. truncatum* infected as nymphae, and adult *H. m. rufipes* transmitted infection to a sheep. No evidence of transovarial transmission was found in larval progeny of ticks exposed to CCHF virus as adults on sheep and cattle or as immatures on scrub hares.

INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne viral zoonosis which is distributed throughout Africa and Eurasia and is being increasingly recognized as a cause of human disease in Africa [1, 2]. The ecology of the virus appears to be complex and the manner in which reservoir hosts and ticks interact in nature to maintain enzootic CCHF is unclear. Human infection acquired by tick bite is most often associated with ticks of the genus *Hyalomma*, which are considered to

* Present address: Department of Medical Microbiology, University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG
† Reprint requests to: Professor R. Swanepoel, National Institute for Virology, Private Bag X4, Sandringham 2131, Republic of South Africa

be the principal vectors, but the virus has been isolated from 30 tick species of 7 genera [1, 3–5) and experimental studies have shown viral replication and horizontal and/or vertical transmission in ixodid ticks of the genera *Hyalomma*, *Rhipicephalus*, *Dermacentor* and *Amblyomma* [1, 6–8]. A large number of vertebrate species have been implicated as potential reservoirs. In southern Africa serological surveys indicate that many wild and domestic animals which act as hosts to infected ticks are susceptible to infection [2, 9] and experimental studies have shown that cattle, sheep, scrub hares, guinea fowl, and several wild rodent species develop viraemia when infected [10–13]. In order to assess the role which viraemic vertebrates play as amplifying hosts of the virus and to relate the results of experimental studies to conditions in the field, it is necessary to obtain information on the threshold levels of viraemia necessary for infection of different tick species. In this paper we report the results of attempts to infect several African tick species by feeding on viraemic vertebrates.

MATERIALS AND METHODS

Virology and serology

The inoculum was a clarified 10% brain suspension from infant mice infected with strain SPU 4/81 of CCHF virus. This had been isolated from a fatal human case in 1981 [5]. The virus was used after 2 cell culture and 3–4 mouse brain passages and had a titre of $10^{6\cdot7-7\cdot0}$ mouse LD 50/ml. Presence of virus in blood or tick extracts was determined by intracerebral inoculation of litters of day-old mice. The mice were observed for 14 days and deaths confirmed as specific by indirect immunofluorescence (IF) tests on impression smears of brain as described previously [14].

Pools of ticks were homogenized with a pestle and mortar to approximately 10% weight/volume in cell culture medium containing antibiotics. The suspensions were centrifuged at 10000 r.p.m. for 10 min and the supernatant assayed in mice for presence of virus. Sera were tested for antibody by IF or reversed passive haemagglutination-inhibition (RPHI) as described previously [9, 15].

Vertebrates and ticks

The mammals used as sources of virus were cattle, sheep, scrub hares (*Lepus saxatilis*), white-tailed rats (*Mystromys albicaudatus*) and guinea-pigs (Duncan-Hartley strain). Scrub hares were captured near Pafuri in the Kruger National Park, Transvaal, and white-tailed rats were obtained from a laboratory colony maintained at the National Institute for Virology. Sheep and cattle were reared in tick-free stables at the Veterinary Research Institute (VRI). Sheep, cattle, and scrub hares were bled and tested for presence of CCHF antibody prior to the experiments.

Hyalomma marginatum rufipes, *H. truncatum*, *Rhipicephalus evertsi evertsi*, *R. e. mimeticus*, *R. appendiculatus*, *R. simus*, *Amblyomma hebraeum* and *Boophilus decoloratus* ticks were obtained from colonies maintained at the Tick Research Unit of the VRI. Sample numbers of adult ticks of each species were tested for presence of virus by inoculation of newborn mice prior to the experiments.

Experimental procedures

Animals were inoculated subcutaneously or intravenously (guinea-pigs) with 0·2 to 0·5 ml of virus suspension containing $10^{6\cdot0-6\cdot7}$ mouse LD 50. With the exception of white-tailed rats, all animals were bled daily for determination of viraemia and antibody. Ticks were fed in bags on sheep and cattle, in capsules affixed to the shaved skin of guinea-pigs, and under velcro fabric fastened round the thorax of white-tailed rats, but were allowed to feed freely on scrub hares. The numbers and species of ticks used in each experiment are indicated in the results. Experiments on sheep and cattle were conducted mainly using adult ticks, but some immature ticks were also fed on sheep. The ticks were placed on the host in groups at intervals which ranged from several days before infection to the day of infection so that each species would ingest viraemic blood during early and late periods of tick feeding. In experiments with guinea-pigs and white-tailed rats, larval ticks were placed on the host on the day of infection.

In an initial experiment with scrub hares, one hare (no. 1) was infected with CCHF virus while being fed on by wild ticks present on the animal when captured. Hares 3 and 4 were held for 3 months prior to infection and were free of wild ticks before the start of the experiments. On these animals, larvae of each of the two host tick species used were fed in two groups so that those in one group fed as larvae and the other as nymphae on the same viraemic animal. In order to determine whether trans-stadial and vertical transmission occurred, adult ticks that moulted from potentially infected immature forms were allowed to feed on susceptible sheep. The sheep were then tested retrospectively for CCHF infection by IF antibody test 28 days after infestation.

After feeding, ticks were either frozen immediately at −70 °C for subsequent virus isolation or held at 25 °C and 80% relative humidity for moulting and egg laying. Female ticks were held individually while egg laying proceeded except for *Boophilus decoloratus*, which were held in groups of five. Ticks were tested in pools for virus content at varying stages after drop-off from the host. The number of ticks in each pool varied in size according to the number of ticks available in each experiment. First generation larval progeny of individual female ticks were tested for virus in pools of approximately 400.

RESULTS

The daily titres of viraemia in each of the animals which were used as virus donors for feeding ticks are presented in Table 1. Maximum mouse LD 50 titres ranged from $10^{2\cdot5-3\cdot2}$ LD 50/ml in sheep, $10^{1\cdot5-2\cdot7}$ LD 50/ml in cattle, $10^{1\cdot9-2\cdot7}$ LD 50/ml in guinea-pigs and $10^{2\cdot6-4\cdot2}$ LD 50/ml in scrub hares. Viraemia was not monitored in white-tailed rats. In previous experiments white-tailed rats were found to develop viraemia between days 1 and 6 after infection, with all inoculated animals viraemic on days 4 and 5 with a mean titre of $10^{2\cdot6}$ LD 50/ml (range $10^{1\cdot6-3\cdot7}$ LD 50/ml) [10]. In the present experiments, infection in white-tailed rats was confirmed by detection of IF antibody at titres of 256–512 day 28 after inoculation.

During the experiment all three sheep and one of the cattle (no. 1230) developed

Table 1. *Daily viraemia (log_{10} mouse LD 50/ml) in animals used as hosts for feeding ticks*

Species	Days after infection									
	1	2	3	4	5	6	7	8	9	10
Sheep 73	—*	—	—	1·5	1·9	2·2	2·9	—	—	—
Sheep 74	—	3·2	2·6	2·6	3·1	1·4	—	—	—	—
Sheep 43	—	1·5	2·5	1·4	2·1	2·0	—	—	—	—
Cow 30	—	—	—	—	2·0	2·7	2·0	1·3	—	—
Cow 34	—	1·5	—	—	—	1·5	—	—	—	—
Guinea-pig 13	1·8	1·7	—	2·0	1·3	—	—	—	—	—
Guinea-pig 15	1·5	1·8	1·7	1·6	1·9	—	—	—	—	—
Guinea-pig 16	1·7	1·8	1·8	2·7	2·1	—	—	—	—	—
Scrub hare 1	2·2	1·5	2·7	1·9	1·5	—	—	—	—	—
Scrub hare 3	1·7	3·7	3·2	2·8	4·2	1·8	2·1	dead		
Scrub hare 4	—	2·6	2·1	—	1·8	1·5	—	—	nd†	nd

* —, less than 1·3 log mouse LD 50/ml.
‡ nd, not done.

clinical signs consistent with those of sweating sickness (a tick toxicosis transmitted by *H. truncatum*). The signs included pyrexia, profuse salivation, trembling, diarrhoea, anorexia, conjunctivitis and hyperaemia of the mucous membranes. In addition, sheep 6274 developed paralysis and weakness caused by *R. e. evertsi* and was killed on day 10 post infection when the majority of ticks had completed feeding. The other animals recovered uneventfully.

The tick species fed on sheep were adult *A. hebraeum*, *H. m. rufipes*, *H. truncatum*, *R. e. evertsi*, *R. appendiculatus* and *R. simus* and larval or nymphal *A. hebraeum*, *R. e. evertsi* and *R. simus*. From the 3 sheep a total of 448 adult ticks in 159 pools and 1147 immature ticks in 57 pools in the engorged state after feeding were tested for virus. A further 136 female ticks were tested after oviposition in 30 pools and 127 batches of larvae which hatched from the eggs of these females were tested in separate pools. Finally, 2367 adult ticks which had moulted from engorged immatures were tested in 70 pools. In sum, therefore, 443 pools of ticks which fed on sheep were tested, none of which yielded CCHF virus.

The results of attempts to isolate CCHF virus from each tick species which fed on the two cattle are shown in Table 2. Of the ticks which fed on animal no. 1230 virus was isolated from 6 of 83 pools tested at drop-off and from 3 of 87 pools of female ticks tested after oviposition, but was not isolated from 74 pools of larvae which hatched from eggs laid by the females. The ticks positive for virus from this animal were all female. Drop-off positives included three pools consisting of single *H. truncatum*, single *R. appendiculatus* and *R. e. evertsi* and one pool of two *B. decoloratus*. All three of the positive pools from females after oviposition consisted of single *H. truncatum*. Of the ticks which fed on animal no. 1234, virus was isolated from 4 of 69 pools tested at drop-off but not from 50 pools of females tested after oviposition or from 49 pools of their larval progeny. The positive pools included three pools of single *A. hebraeum* females and one pool of 10 *R. e. evertsi* males. In total, CCHF virus was isolated from five of the six tick species which fed on the cattle. Titres of virus ranged from $10^{1·8–2·7}$ LD 50/ml (mean $10^{2·2}$ LD 50/ml)

https://doi.org/10.1017/S0950268800048524 Published online by Cambridge University Press

Table 2. *Isolation of CCHF virus from ticks fed on viraemic cattle*

Animal	Tick species	Engorged		Postoviposition	
		Males	Females	Females	Larvae
1230	*A. hebraeum*	19/2/0*	4/4/0	6/6/0	4/0†
1230	*B. decoloratus*	—	171/31/1	137/31/0	31/0
1230	*H. m. rufipes*	2/1/0	—	2/2/0	1/0
1230	*H. truncatum*	40/2/0	16/16/3	14/14/3	7/0
1230	*R. appendiculatus*	17/3/0	11/11/1	17/17/0	14/0
1230	*R. e. evertsi*	25/2/0	11/11/1	17/17/0	17/0
		103/10/0	213/73/6	193/87/3	74/0
1234	*A. hebraeum*	33/3/0	7/7/3	9/9/0	9/0
1234	*B. decoloratus*	—	144/31/0	23/12/0	12/0
1234	*H. m. rufipes*	2/1/0	1/1/0	3/3/0	3/0
1234	*H. truncatum*	2/1/0	9/9/0	9/9/0	8/0
1234	*R. appendiculatus*	7/1/0	11/11/0	13/13/0	13/0
1234	*R. e. evertsi*	13/3/1	1/1/0	4/4/0	4/0
Subtotal		57/9/1	173/60/3	61/50/0	49/0
Total		160/19/1	386/133/9	254/137/3	123/0

* No. ticks/no. pools/no. of pools virus positive.
† No. pools/no. positive.

Table 3. *Isolation of CCHF virus from ticks fed as larvae or nymphae on viraemic scrub hares*

Hare no.	Tick species	Group*	Tested as:†			
			Larvae	Nymphae	Adults	Total
3	*R. e. evertsi*	A	784/16/0‡	—	—	0/16§
3	*R. e. evertsi*	B	—	121/8/0	73/12/0	0/20
3	*H. truncatum*	A	136/3/2	—	—	2/3
3	*H. truncatum*	B	—	51/4/2	16/5/2	4/9
3	*H. m. rufipes*	A	150/3/3	—	—	3/3
3	*H. m. rufipes*	B	—	39/3/2	39/12/5	7/15
4	*R. e. evertsi*	A	147/4/0	49/8/0	73/11/0	0/23
4	*R. e. evertsi*	B	—	75/7/0	55/12/0	0/19
4	*H. truncatum*	A	65/2/0	53/10/0	39/8/0	0/20
4	*H. trunctatum*	B	—	9/3/0	15/4/0	0/7
4	*H. m. rufipes*	A	80/2/0	93/17/0	90/15/0	0/34
4	*H. m. rufipes*	B	—	38/8/1	72/17/1	2/25

* Group A ticks fed as larvae during the period of viraemia. Group B ticks fed as nymphae.
† Larvae and nymphae tested engorged. Adults were tested approximately 1 month after moulting.
‡ No. ticks/no. pools/no. of pools virus positive.
§ No. pools virus positive/no. tested.

in the 10 drop-off pools and from $10^{1\cdot3-2\cdot2}$ LD 50/ml (mean $10^{1\cdot7}$ LD 50/ml) in the three females tested after oviposition.

Of the ticks that fed on 3 guinea-pigs, a total of 383 *A. hebraeum*, 348 *H. truncatum* and 68 *R. e. mimeticus* engorged larvae were tested after drop-off in 28 pools. A further 257 *A. hebraeum* and 609 *H. truncatum* nymphae were tested after

Table 4. *Mean titres of CCHF virus (log_{10} mouse LD 50/ml) in pools of various stages of* H. m. rufipes *and* H. truncatum *ticks tested after feeding on viraemic scrub hares*

Stage	*H. m. rufipes*	*H. truncatum*	Combined (range)
Engorged larvae	2·4 ($n = 3$)*	1·3 ($n = 2$)	2·0 (1·3–3·8)
Engorged nymphae	2·4 ($n = 3$)	2·9 ($n = 2$)	2·4 (1·3–4·5)
Unfed adults	2·8 ($n = 6$)	3·5 ($n = 3$)	3·0 (2·2–4·2)
Fed males	2·8 ($n = 4$)	—	2·8 (2·2–3·6)

* n, no. of pools positive for virus.

moulting in 7 and 12 pools respectively. None of the pools yielded CCHF virus. Similarly, no virus was isolated from 98 *A. hebraeum*, 60 *H. truncatum* or 5 *R. e. mimeticus* engorged larvae which were tested in 19 pools after drop off from 4 white-tailed rats, nor from 42 nymphs and adults of these species which were tested in 9 pools after moulting.

Of the wild ticks that fed on hare 1, CCHF virus was not isolated from 8 pools comprising 71 adults of 5 species which dropped as engorged nymphae prior to inoculation of the hare. Virus was, however isolated from 1 of 8 pools of adult ticks which dropped from the hare as nymphae on days 1–9 after infection. The virus positive pool consisted of 7 *H. truncatum* which dropped on days 6 and 7, while the negative pools contained 45 *H. truncatum*, 4 *R. e. evertsi* and 1 *R. appendiculatus*. The titre of virus in the positive pool was $10^{2·9}$ LD 50/ml. The results of attempts to isolate CCHF virus from the three tick species which fed on hares 3 and 4 are presented in Table 3. Of the ticks that fed as larvae on hare 3, (maximum viraemia $10^{4·2}$ LD 50/ml) virus was isolated from 2 of 3 pools of *H. truncatum* and 3 of 3 pools of *H. m. rufipes* larvae at drop off, but not from 16 pools of *R. e. evertsi*. Hare 3 was killed on day 8 following an injury sustained during bleeding and all ticks still feeding on that day were removed. Further engorged larval ticks which were held for moulting died. Of the ticks which fed as nymphae and were tested at drop off, virus was isolated from 2 of 4 pools of *H. truncatum* and from 2 of 3 pools of *H. m. rufipes*, but not from 8 pools of *R. e. evertsi*. Similarly, after moulting to adults, virus was isolated from 2 of 5 pools of *H. truncatum* and from 5 of 12 pools of *H. m. rufipes*, but not from 12 pools of *R. e. evertsi*. Of the ticks that fed on hare 4, (maximum viraemia $10^{2·7}$ LD 50/ml) virus was not isolated from ticks of all 3 species that fed as larvae when tested as engorged nymphae or after moulting to adults. Of the ticks that fed as nymphae, virus was isolated from 1 of 8 pools of engorged *H. m. rufipes*, but not from pools of *H. truncatum* and *R. e. evertsi*. After moulting to adults, virus was isolated from 1 of 17 pools of *H. m. rufipes* but not from pools of the other species.

Potentially infected *H. m. rufipes* and *H. truncatum* adults that fed as nymphae on hare 3 were fed 2 months after moulting in separate groups on 2 sheep. CCHF antibody was not detected by IF in the first sheep that fed 12 male and 9 female *H. truncatum*, nor was virus isolated from 3 pools of fed male ticks, 9 females after oviposition or 8 pools of larvae derived from these females. The other sheep, which was fed upon by 11 male and 10 female *H. m. rufipes*, seroconverted and on day 28 the IF antibody titre was 256. CCHF virus was isolated from 4 of 6 pools of fed

males, but not from 1 engorged female, 9 females after oviposition or 8 pools of larvae derived from these females.

The viral titres of positive pools of *H. m. rufipes* and *H. truncatum* that fed on scrub hares are presented in relation to life-cycle stage in Table 4. Titres ranged from $10^{1\cdot3-4\cdot5}$ LD 50/ml, with the overall mean titres in newly emerged adults and fed male ticks being higher than those for engorged larvae and nymphae.

DISCUSSION

The concept of threshold levels of viraemia for arthropod infection was first established by Chamberlain and others in studies of mosquito transmission of Western and Eastern Equine Encephalitis viruses [16] and has since been extensively studied in several mosquito-virus systems [17]. In contrast to mosquitoes which feed and digest the bloodmeal rapidly, ticks feed over a period of several days during which there may be considerable change in the virus titre in the blood. Consequently, it is more difficult to quantify threshold levels of viraemia for ticks with any accuracy although it is generally accepted that the probability of viral transmission to feeding ticks is directly proportional to the intensity of viraemia in the host [18, 19]. Recently, however, Jones and colleagues [20] have shown transmission of Thogoto virus to uninfected *Rhipicephalus appendiculatus* which were co-fed with infected ticks on hosts in which viraemia was undetectable. This 'non-viraemic' transmission was enhanced by factors present in tick salivary glands [21]. Whether such transmission occurs with CCHF or the related Dugbe virus has not been conclusively established [7, 22]. Threshold viraemias of between $10^{2\cdot0-4\cdot7}$ LD 50/ml have been reported for various tick species infected with Colorado tick fever, Russian spring-summer encephalitis and louping ill viruses [18, 19, 23]. In the only reported study to determine the threshold viraemia of CCHF virus, Zgurskaya and colleagues [24] established that larval *H. m. marginatum* fed on hares and rabbits became infected at viraemic titres of $10^{4\cdot4-5\cdot3}$ LD 50/ml but not at titres of $10^{2\cdot4-3\cdot8}$ LD 50/ml. As regards larval ticks, the present results are in agreement with Zgurskaya and colleagues [24]. Larval ticks of three species were not infected after feeding on viraemic guinea-pigs, white-tailed rats or scrub hares with maximum viraemias of $10^{1\cdot3-2\cdot6}$ LD 50/ml, but *H. m. rufipes* and *H. truncatum* larvae became infected after feeding on a scrub hare with viraemia of $10^{4\cdot2}$ LD 50/ml. However, in other experiments, CCHF virus was transmitted to nymphal *H. m. rufipes* and *H. truncatum* which fed on scrub hares with maximum viraemias of $10^{2\cdot6-2\cdot7}$ LD 50/ml and to adult ticks of five species which fed on cattle with maximum viraemia of $10^{1\cdot5-2\cdot7}$ LD 50/ml. These results suggest that there are differences in the susceptibility of each of the three tick life cycle stages to ingested CCHF virus and that threshold values of infection are lower for nymphae and adult ticks than for larvae. Previously Beasley and colleagues [19] reported that the proportion of louping ill virus infected *Ixodes ricinus* nymphae was greater than that of larvae after co-feeding on viraemic hosts, while Blackburn and colleagues [25] obtained infection rates of 3·5% and 57·5% respectively in larval and nymphal *Haemaphysalis leachii* which were fed on hamsters infected with West Nile virus. The greater susceptibility of adults and nymphae to CCHF virus may be related

to the fact that, being of greater size than larvae, they ingest a larger volume of blood and consequently a larger virus dose. Given these results, it was surprising that adult ticks were not infected after feeding on sheep with maximum viraemia of $10^{2\cdot5-3\cdot2}$ LD 50/ml. Possibly the high temperatures in the animals caused by concurrent sweating sickness inhibited the establishment of infection in the ticks or was detrimental to the survival of the virus in infected ticks.

In previous studies to determine the role of cattle as amplifying hosts of CCHF, Causey and colleagues [12] observed infection of a single *H. m. rufipes* female that fed on a viraemic calf, while Zarubinsky and colleagues [13] failed to infect adult ticks of three species fed on experimentally infected calves. The results of the present study demonstrate that adult ixodid ticks of several species can acquire CCHF virus infection by feeding on viraemic cattle. It is unknown whether the virus isolated from adult ticks after feeding represented virus replication in the tick, or merely passive survival of virus ingested in the bloodmeal. Titres of virus in these adult ticks were low in comparison to the unfed adults and fed males infected as nymphae on hares (Table 4). However, we previously observed a progressive decline in ability to isolate CCHF virus from adult ticks (derived from intracoelomically infected nymphs) of three species after feeding on sheep [8]. The presence of virus in three *H. truncatum* females tested after oviposition (approximately 1 month after feeding) suggests that replication may have taken place in some infected ticks.

The majority of the virological surveys for CCHF reported in the literature have been conducted in adult ticks collected from domestic animals [1], and given the low threshold of infection for adult ixodids observed here, it is probable that a large proportion of the reported virus isolations in the literature were from ticks which had been infected by ingestion of viraemic blood. This is implicit in the report of Pak [26] who observed that the isolation rate of CCHF in Tadzhikistan, USSR, was 18 times higher in *Hyalomma anatolicum anatolicum* removed from cattle than in those collected unfed.

As ixodid ticks feed only once in each instar, adult ticks infected by feeding on viraemic vertebrates can be of importance in the virus cycle only if they transmit virus transovarially. In the present study, none of the female ticks exposed to CCHF virus by feeding on sheep and cattle transmitted infection to the larval progeny, even though virus was isolated from three *H. truncatum* females after oviposition. These results indicate that isolation of CCHF virus from a tick species taken from domestic animals cannot be used as a criterion for assessment of that species as a virus vector. However, regardless of whether they transmit virus transovarially, adult ticks which become infected by feeding may be an important source of human infection if removed by hand or squashed.

The results of the present study confirm that the scrub hare, which is among the most important hosts of immature *Hyalomma* ticks [27, 28], must be regarded as an amplifying host of CCHF virus in Africa. Immature *H. m. rufipes* and *H. truncatum* which became infected by feeding upon scrub hares transmitted virus trans-stadially and *H. m. rufipes* adults infected in this manner transmitted virus to a sheep. In contrast to the observations in adult ticks, there were differences in the susceptibility to CCHF infection of immature tick species which fed on viraemic scrub hares. In these experiments virus was transmitted to larval and

nymphal *H. m. rufipes* and *H. truncatum* but not to *R. e. evertsi*, although *R. e. evertsi* adults of the same strain became infected by feeding on viremic cattle. These results indicate that there are differences in the threshold values of infection for immature stages of ixodid tick species and the implication is that threshold levels for *Hyalomma* species may be lower than for other ixodid ticks. If this were true, it may in part explain why *Hyalomma* species are the principal vectors of human CCHF infection.

Several previous studies demonstrated vertical transmission of CCHF virus in ticks of the genera *Hyalomma*, *Rhipicephalus* and *Dermacentor*, [1] but these results have not been confirmed in more recent studies [7, 8]. In this study, we again failed to observe transovarial transmission in ticks infected or exposed to CCHF virus by feeding in the immature or adult stage on scrub hares, sheep or cattle. As previously discussed [8], the genetic or other factors which determine vertical transmission of tick-borne viruses are poorly understood and so the present results cannot be regarded as evidence that vertical transmission of CCHF virus does not occur in the wild.

In this study we show that cattle and scrub hares can act as amplifying hosts of CCHF virus by viraemic transmission of virus to ticks. The possibility that such transmission may be augmented in the wild by other forms of transmission, such as non-viremic transmission mediated by salivary gland factors [21, 22], cannot be discounted and requires further study.

ACKNOWLEDGEMENTS

The Director, Veterinary Research Institute, Onderstepoort, and the Director General of Health and Population Development have given permission for publication of this article. Financial support was provided by the Medical Research Council and Poliomyelitis Research Foundation of South Africa.

REFERENCES

1. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe and Africa. J Med Entomol 1979; **15**: 307–417.
2. Swanepoel R, Shepherd AJ, Leman PA, et al. Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. Am J Trop Med Hyg 1987; **36**: 120–32.
3. Sureau P, Klein JM, Casals J, et al. Isolement des virus Thogoto, Wad Medani, Wanowrie et de la fievre hemorrhagique de Crimee-Congo en Iran a partir de tiques d'animaux domestiques. Ann Virol 1980; **131**: 185–200.
4. Boiro I, Butenko AM. 1983. Recherches virologiques. Centre Collaborateur OMS de reference et de recherche pour les arbovirus. Institute Pasteur, Dakar: Annual report, 34–9.
5. Swanepoel R, Struthers JK, Shepherd AJ, McGillivray GM, Nel MJ, Jupp PG. Crimean-Congo hemorrhagic fever in South Africa. Am J Trop Med Hyg 1983; **32**: 1407–15.
6. Okorie TG, Fabiyi A. The replication of Congo virus in *Hyalomma rufipes* Koch following intracoelomic inoculation. Vet Parasitol 1980; **7**: 369–74.
7. Logan TM, Linthicum KJ, Bailey CL, Watts DM, Moulton JR. Experimental transmission of Crimean-Congo hemorrhagic fever virus by *Hyalomma truncatum* Koch. Am J Trop Med Hyg 1989; **40**: 207–12.
8. Shepherd AJ, Swanepoel R, Cornel AJ, Mathee O. Experimental studies on the replication and transmission of Crimean-Congo hemorrhagic fever virus in some African tick species. Am J Trop Med Hyg 1989; **40**: 326–31.

9. Shepherd AJ, Swanepoel R, Shepherd SP, McGillivray GM, Searle LA. Antibody to Crimean-Congo hemorrhagic fever virus in wild mammals from southern Africa. Am J Trop Med Hyg 1987; **36**: 133–42.
10. Shepherd AJ, Leman PA, Swanepoel R. Viremia and antibody response of small African and laboratory mammals to Crimean-Congo hemorrhagic fever virus infection. Am J Trop Med Hyg 1989; **40**: 541–7.
11. Shepherd AJ, Swawnepoel R, Leman PA, Shepherd SP. Field and laboratory investigation of Crimean-congo haemorrhagic fever virus (Nairovirus: family Bunyaviridae) infection in birds. Trans Roy Soc Trop Med Hyg 1987; **81**: 1004–7.
12. Causey OR, Kemp GE, Madbouly MH, David-West TS. Congo virus from domestic livestock, African hedgehog and arthropods in Nigeria 1970; Am J Trop Med Hyg 1970; **19**: 846–50.
13. Zarubinsky VY, Kondratenko VF, Blagoveshcenskaya NM, Zarubina LV, Kuchin VV. Susceptibility of calves and lambs to Crimean hemorrhagic fever virus. Tezisy Dokl Vses Konf Prirod Ochag Bolez Chelov Zhivot (Omsk) 1976; 130–1.
14. Shepherd AJ, Swanepoel R, Gill DE. Evaluation of enzyme-linked immunosorbent assay and reversed passive hemagglutination for detection of Crimean-Congo hemorrhagic fever virus antigen. J Clin Microbiol 1988; **26**: 347–53.
15. Swanepoel R, Struthers JK, McGillivray GM. Reversed passive hemagglutination and inhibition with Rift Valley fever and Crimean-Congo hemorrhagic fever viruses. Am J Trop Med Hyg 1983; **32**: 610–17.
16. Chamberlain RW, Sikes RK, Nelson DB, Sudia WB. Studies on the North American arthropod-borne encephalitides. VI. Quantitative determination of virus-vector relationships. Am J Hyg 1954; **60**: 278–85.
17. Hardy JL, Houk EJ, Kramer LD, Reeves EC. Intrinsic factors affecting vector competence of mosquitoes for viruses. Ann Rev Entomol 1983; **28**: 229–62.
18. Rehacek J. Development of animal viruses and rickettsia in ticks and mites. Ann Rev Entomol 1965; **10**: 1–24.
19. Beasley SJ, Campbell JA, Reid HW. Threshold problems in infection of *Ixodes ricinus* with the virus of louping ill. In: Wilde JKH ed. Tickborne diseases and their vectors. Edinburgh: Centre for Tropical Veterinary Medicine 1978; 497–500.
20. Jones LD, Davies CR, Steele GM, Nuttal PA. A novel mode of arbovirus transmission involving a nonviremic host. Science 1987; **237**: 775–7.
21. Jones LD, Hodgson E, Nuttal PA. Enhancement of virus transmission by tick salivary glands. J gen Virol 1989; **70**: 1895–8.
22. Linthicum KJ, Logan TM, Bailey CL, Watts DM, Dohm DJ. Experimental infection of six species of ixodid ticks with Dugbe virus (Family Bunyaviridae, Genus *Nairovirus*). Am J Trop Med Hyg 1989; **40**:410–7.
23. Burgdorfer W, Varma MGR. Trans-stadial and transovarial development of disease agents in arthropods. Ann Rev Entomol 1967; **12**: 347–76.
24. Zgurskaya GN, Berezin VV, Smirnova SE. Threshold levels of blood infectiousness for *Hyalomma p. plumbeum* ticks during viremia in hares and rabbits caused by CHF virus. Tezisy Konf Vop Med Virus (Moscow) 1975; 291–2.
25. Blackburn NK, Shepherd AJ, Paterson B, Besselaar TG. Susceptibility of the dog tick *Haemaphysalis leachii* Audoin (Acarina: Ixodidae) to West Nile virus. J Ent Soc Sth Afr 1990; **53**: 11–6.
26. Pak TP. Contribution to the question of development of the Crimean hemorrhagic fever noso-distribution area. Tezisy Konf Vop Med virus (Moscow) 1975; 335–7.
27. Horak IG, MacIvor KMdeF. The scrub hare, a reliable indicator of the presence of *Hyalomma* ticks in the Cape Province. J S Afr Vet Assoc 1987; **58**: 15–9.
28. Rechav Y, Zeederberg ME, Zeller DA. Dynamics of African tick (Acari: Ixodoidea) populations in a natural Crimean-Congo hemorrhagic fever focus. J Med Entomol 1987; **23**: 484–88.