

Rickettsial diseases - Animals - Ethiopia

Evidence of Rickettsial Disease Agents in Ticks from Ethiopian Cattle*

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Evidence has recently been accumulating that domestic animals may play an ancillary role in rickettsial zoonoses. In particular, attention has been focused on the activity of Rickettsia prowazekii in Egyptian and Ethiopian livestock and their ticks. An attempt has now been made to confirm previous findings of R. prowazekii in the sera of zebus, sheep and goats in Ethiopia, which brought epidemic typhus into the category of a zoonosis. This attempt was not successful, but tests did indicate that some ticks were infected with R. conori (boutonneuse fever or tick-borne typhus) and Coxiella burneti (Q fever), this being the first evidence for the existence of these agents in Ethiopia.

Antibodies against R. conori were found in significant numbers in the sera of sheep and goats from one locality, but Q-fever antibodies were surprisingly rare.

In recent years evidence has been accumulating that typhus fever, in addition to being transmitted through the usual cycles involving man, whether the causative agent comes from an epidemic or a murine source, is also implicating domestic animals. Reiss-Gutfreund (1955, 1956, 1961) demonstrated this in her studies of Ethiopian livestock and their ticks. Briefly, she not only found antibodies against *Rickettsia prowazekii* and OX₁₉ agglutinins in the sera of a proportion of zebus, sheep and goats, but, what is more important, she isolated proven strains of the organism from two goats, two sheep, and one patient, as well as four strains from *Amblyomma variegatum* and four from *Hyalomma rufipes* col-

lected from zebus and goats.⁵ Imam (1963) has obtained serological evidence of antibodies against the epidemic and murine forms of typhus in a significant percentage of Egyptian camels, goats and cattle.

Further studies in Ethiopia are the subject of the present paper.

COLLECTION OF MATERIALS FROM ETHIOPIAN LIVESTOCK

In December 1962 and March 1963, one or more of the authors collected ticks from cattle in central and southern Ethiopia. Forty tick lots, comprising five genera and 11 species, mostly adults, were taken. The composition of these collections is given in Table 1; in all, 3723 ticks were taken for testing, together with a few unrecorded *Haemaphysalis leachii*, which were fed and kept for oviposition.

Amblyomma cohaerens and *A. variegatum* were the most abundant species found on the cattle. The fact that there were many more males than females of these species and of *A. gemma* suggests that these species had passed their peak occurrence.

In addition, sera from the following animals were collected at the Addis Ababa abattoir: 10 goats and

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⁵ Since this report was presented at the Congress, Reiss-Gutfreund has again made a critical isolation of the agents of epidemic and murine typhus from ticks taken from Ethiopian cattle and this isolation has been confirmed at the Rocky Mountain Laboratory.

TABLE 1
SPECIES OF TICKS COLLECTED FROM ETHIOPIAN CATTLE

Species	Females		Males (and nymphs)		Total
	Dec. 1962	March 1963	Dec. 1962	March 1963	
<i>Amblyomma</i>					
<i>cohaerens</i>	146	298	429	988	1 861
<i>gemma</i>	4	1	377	11	393
<i>lepidum</i>	—	—	3	3	6
<i>variegatum</i>	17	55	144	310 (10)	526 (10)
<i>Boophilus</i>					
<i>decoloratus</i>	120	56	1	2 (3)	179 (3)
<i>Hyalomma</i>					
<i>rufipes</i>	29	1	43	24	97
<i>truncatum</i>	31	14	38	19	102
<i>Rhipicephalus</i>					
<i>evertsi</i>	98	18	126 (2)	48 (4)	290 (6)
<i>simus</i>	70	5	99	6	180
<i>pulchellus</i>	15	10	17	28	70
<i>Haemaphysalis</i>					
<i>leachi</i>	few	—	—	—	

six sheep from Adama, and 29 oxen from Arussi, Bale, Shoa and Ambo. It was, unfortunately, not practicable in the limited time to bleed the wandering cattle in these Ethiopian localities, where appropriate antibody reactors to the tick-borne agents discussed below might have been discovered.

LABORATORY STUDIES

Methods

The tick and serum samples were transported by air to the Rocky Mountain Laboratory, where tick pools, sorted according to species, were triturated in serum-saline, an antibiotic being added to reduce contamination. Each pool was divided: one-half for immediate injection into male guinea-pigs, the other frozen for future reisolation needs. Unusually heavy contamination of the initially tested tick suspensions necessitated doubling the amount of penicillin to 4000 units and of streptomycin to 1000 units per 2.7 ml of thawed aliquots when these were retested. Transfers from test animals were made from blood or tissues during any febrile episodes after suitable incubation periods. Animals that

recovered were bled for antibody assay and challenged with virulent Rocky Mountain spotted fever rickettsiae, if this procedure was indicated by positive results of serological tests carried out in the unit headed by Dr David B. Lackman at the Rocky Mountain Laboratory.

Results of tick tests

Evidence of infection with *Rickettsia conori* and *Coxiella burneti* is presented in Table 2. To avoid contamination in the laboratory, no attempt was made to maintain and type a strain of *Salmonella* that was isolated from *Hyalomma rufipes* taken from cattle at Lake Koka.

The Simko isolate of *Rickettsia conori* from fed *Rhipicephalus simus* (Lot 10, Table 2), Lake Koka, was shown to immunize guinea-pigs against challenge with virulent Rocky Mountain spotted fever and to be antigenically related to other spotted-fever-group antigens. This isolate was further shown, by the mouse-toxin neutralization tests of Dr John Bell, to be specifically related to a strain of Kenya tick typhus isolated at the Rocky Mountain Laboratory some years ago from *Haemaphysalis leachii* obtained

TABLE 2
RICKETTSIAL AGENTS IN TICKS FROM ETHIOPIAN CATTLE

Disease	Agent	Tick species	No. and sex of ticks	Lot and source	Notes ^a
Boutonneuse fever	<i>Rickettsia conori</i>	<i>Rhipicephalus simus</i>	15 males 15 females	No. 10, Lake Koka, Dec. ^b	Ticks reattached on guinea-pigs; Simko strain; good RMsf-group antigen from yolk sacs.
		<i>Amblyomma variegatum</i>	4 males injected	No. 9, Nazareth, Dec.	Test guinea-pigs had low fever; transfer guinea-pigs immune to RMsf group; Q-fever antibodies.
		<i>Amblyomma cohaerens</i>	42 males and females injected	No. 40, Awash River, March	Strain isolated, three passages; immune to challenge; CF antibodies.
72 males and females injected	No. 32, Awash River, March		Test guinea-pigs afebrile; immune to challenge.		
Q fever	<i>Coxiella burneti</i>	<i>Amblyomma variegatum</i>	21 males and 7 females injected	Pool 4, 6, 11: Lake Koka No. 14: Akaki, Dec.	Original guinea-pigs afebrile, CF-positive, immune; isolate from frozen aliquot into guinea-pigs transferred to chick embryos.
		<i>Hyalomma truncatum</i>	6 males 5 females	No. 12, Lake Langano, Dec.	Test guinea-pigs afebrile; immune to challenge.

^a No typhus antibodies were observed in test animals.

^b An isolate of *Salmonella*, not typed, was isolated from a guinea-pig injected with a suspension of 10 males and four females of *Hyalomma rufipes* from the same lot.

in Kenya. The Simko strain has been maintained in this laboratory because it has provided a good spotted-fever-group antigen and is well adapted to growth in yolk sacs of chick embryos. In subsequent serological tests, this antigen proved more sensitive than standard Rocky Mountain spotted fever and rickettsialpox antigens for determining antibody titres in animals that had recovered from *Rickettsia conori* infection.

Not only was the Awash River isolate (Lot 40, Table 2) carried for three guinea-pig passages, but a yolk-sac culture produced characteristic infection, accompanied by scrotal swelling, in two guinea-pigs. It also produced immunity to Rocky Mountain spotted fever.

Because of sporadic fevers following injection of a pool of *A. variegatum* (Lot 9, Table 2) from Nazareth, one of two test guinea-pigs was sacrificed on the ninth day and suspensions of brain, spleen and tunica were passaged through further animals. These secondary test animals remained afebrile, but showed Q-fever complement-fixation (CF) antibodies 38 days later and were subsequently immune to challenge with virulent Rocky Mountain spotted fever. They also carried antibodies in low titre against the Simko strain before challenge.

The two Q-fever-positive tests (Table 2) obviously concerned *C. burneti* of such low virulence that

inoculation of guinea-pigs produced only immunity. Retesting of a frozen aliquot of one tick suspension (Pool 4, Table 2) in cortisone-treated guinea-pigs provided a strain of constant low virulence in subsequent passages. In our experience, this is not unusual with *C. burneti* from animal sources.

In addition to the evidence for *Rickettsia conori* in ticks given in Table 2, several other tests suggest that this organism has a wide distribution in Ethiopian ticks. All guinea-pigs developed low-level CF antibodies against a Rocky-Mountain-spotted-fever-soluble Simko antigen and, in three instances, against an Indian tick-typhus antigen, in the following feedings: several males and eight females (two tests) of *Rhipicephalus evertsi* and seven females of *R. pulchellus* from Awash Valley, several males and five females of *A. cohaerens* from near Lake Awasa, a pool of several males and four females of *A. variegatum* from four localities, and a pool of 24 males and one female of *Hyalomma marginatum rufipes* from eight localities. Injection of pools of *A. variegatum* from Lake Awasa and Shoshemane, of *A. cohaerens* (three tests) from between Lake Awasa and Lake Langano, and (of special interest) of a pool of 31 females of one-host *Boophilus decoloratus* from between Nazareth and Lake Langano provided similar antibodies. Most of the animals that recovered were partially to completely resistant to

challenge with virulent Rocky Mountain spotted fever, whereas all the control animals developed scrotal reactions and died of characteristic disease.

Results of tests on animal sera

Typhus-group antibodies were not found in any of the 45 livestock samples. Significant spotted-fever-group antibodies occurred in four (66%) sheep and three (30%) goats from Adama, but in no oxen from the other localities. Q-fever antibodies against phase II antigen occurred in only one sheep and two goats, an unexpectedly low percentage in the livestock tested. Psittacosis-group antibodies were found in sera from two oxen from Arussi and in one sheep from Adama.

DISCUSSION

Since the primary purpose of these studies was to try to repeat Reiss-Gutfreund's observations of the involvement of Ethiopian livestock in an epidemic typhus cycle, we were disappointed to find no evidence of *Rickettsia prowazekii* in either the serological or the tick tests. As Imam (unpublished results) has reported, there is evidence for a seasonal variation in the occurrence of typhus antibodies in Egyptian livestock, and this finding, coupled with the need to use nearly double the usual dose of antibiotic to reduce contamination in our tick suspensions, may explain our failure to demonstrate the presence of typhus infection. Furthermore, we have no way of knowing if the well-known cyclic

fluctuations in human outbreaks could also influence the way in which livestock are involved in epidemic typhus.

Though the serological tests are too few to be more than suggestive, it is interesting that *R. conori* is prevalent in sheep and goats in the Adama area but not in oxen from four other localities. It is probably a coincidence that, in similar studies in Ecuador (Philip, unpublished results), 23 of 156 sheep sera, but only one of 151 cattle sera, contained *R. rickettsi* antibodies.

The tick-typhus agent is obviously widely distributed in the Ethiopian tick population. Three species of ticks from cattle in four Ethiopian localities were apparently infected with *R. conori* (Table 2), while serological and immunological evidence (not tabulated) suggests that the experimental feeding of three other species and injection of a fourth produced low-level antibodies, the strongest reaction being against Simko antigen from a local isolate. The infection in *Boophilus decoloratus* is notable. The occurrence of rickettsial antibodies in domestic animals now provides a possible answer to the enigma of several reports of rickettsial infections in the one-host ticks, *Boophilus* sp. (reviewed by Philip, 1963). The most recent review of various ticks as hosts of *R. conori* in southern Europe and the Mediterranean area is that by Giroud et al. (1963).

It does not appear that Q fever is very active in Ethiopian livestock in the areas in which we worked. There is evidence of some mammal involvement in the psittacosis group of diseases.

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RÉSUMÉ

Cette étude a été entreprise en Ethiopie en vue de confirmer la participation du bétail au cycle de transmission du typhus épidémique dû à *Rickettsia prowazeki*.

Au total 3723 tiques appartenant en majorité aux espèces *Amblyomma cohaerens* et *A. variegatum* ont été recueillies sur du bétail dans le centre et le sud du pays.

Réparties en pools, les tiques ont été broyées, mises en suspension et inoculées au cobaye pour identification des souches de rickettsies. L'infection par *R. conori* a été décelée chez *Rhipicephalus simus*, *A. variegatum* et *A. cohaerens*, cependant que *Coxiella burneti*, agent de la fièvre Q était identifié chez *A. variegatum* et *Hyalomma*

truncatum. La présence de *R. prowazeki* n'a pas été démontrée.

D'autre part, la recherche d'anticorps spécifiques a été pratiquée sur 45 échantillons de sérum prélevés à l'abattoir d'Addis-Abéba chez des chèvres, des moutons et des bœufs en provenance de différentes régions du pays.

Aucun anticorps vis-à-vis des antigènes du groupe typhique n'a été décelé, mais en revanche on a noté des titres significatifs d'anticorps vis-à-vis des antigènes du groupe pourpré chez 66% des moutons et 30% des chèvres. Des anticorps vis-à-vis de *Coxiella burneti* n'ont été observés que chez un très petit nombre d'animaux.

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