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Role of Cattle in the Epidemiology of Tick-Bite Fever in Zimbabwe

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Almost 100% of 52 cattle tested from the southern areas of Zimbabwe were found to have antibodies reactive with *Rickettsia conorii* compared with <30% of 120 cattle from the north. Steers artificially infected with *R. conorii* isolated from *Amblyomma hebraeum* were found to show no hematological or biochemical signs of disease but did seroconvert. Clinical signs of infection were restricted to regional lymphadenopathy and dermal erythema, edema, and tenderness at the inoculation site. Rickettsemia was detectable for at least 32 days postinfection. Our findings indicate that cattle could be involved in the transmission of rickettsias by *A. hebraeum* and may serve as a reservoir of human tick-bite fever in southern Africa.

Little is known of the epidemiology of *Rickettsia conorii*, the causative agent of human tick-bite fever in southern Africa. Gear (4) has shown that rodents may play a role in disseminating the disease, and surveys of dogs, both in South Africa (5) and in the Mediterranean (13, 14), have shown a high prevalence of antibodies reactive with *R. conorii*. Whether dogs serve as reservoirs of infection for humans or merely bring infected ticks into contact with humans is not clear (14).

A number of anecdotal reports have implicated *Amblyomma hebraeum* as a vector of tick-bite fever in southern Africa (4, 7, 9). In Zimbabwe, this tick is only found in scattered localities in the north, but is commonly encountered in the south, the area reported to have the highest incidence of human tick-bite fever (9). Since the preferred hosts of the adults, nymphs, and larvae of this tick species are cattle (9), we carried out a survey for antibodies reactive with *R. conorii* in cattle from the north and south of the country. We also investigated the pathogenesis in cattle of a spotted fever group rickettsia isolated from *A. hebraeum* and the role of infected animals in the transmission of these rickettsia.

MATERIALS AND METHODS

Cattle serosurvey. Adult cattle from the south (Mbizi, Tanganda Halt, and Bulawayo) and north (Harare, Copper Queen, Mugunje, Chakonda, and Murewa) of Zimbabwe were bled from the tail vein. The blood samples were centrifuged, and the serum was separated and stored at −20°C. Indirect fluorescent-antibody (IFA) tests were carried out with antigen prepared as described below. Fluorescein-labeled goat anti-bovine immunoglobulin G (IgG) (H + L chains) (Kirkegaard & Perry Laboratories) was used at 1/30 dilution in phosphate-buffered saline. As with serosurveys in other animals (13, 14), a serum titer of ≥1/40 was considered positive.

Rickettsial isolation. Adult male *A. hebraeum* were obtained from a line that had been established from ticks collected from cattle in Mbizi. The line had been maintained on sheep at the Veterinary Research Laboratories, Harare, for about 1 year. Ticks that were positive for rickettsialike organisms (RLO) by the hemolymph test (1) were washed for 5 min each in 10% hydrogen peroxide–70% alcohol and left to dry. They were then triturated in 1.5 ml of ice-cold brain heart infusion broth. The suspension was allowed to settle for 5 min, and the clear supernatant was used for the following procedures.

To establish that the organisms were virulent, 0.1 ml of the supernatant was inoculated intraperitoneally (i.p.) into a male Hartley guinea pig which was subsequently examined daily for temperature elevation and scrotal swelling. After 2 weeks the animal was bled by cardiac puncture and its serum was separated for IFA testing.

RLO were grown in tissue culture by inoculating 0.5 ml of the supernatant into a 25-ml flask containing a confluent layer of Vero cells from which the medium (originally containing penicillin, 100 U/ml; streptomycin, 100 μg/ml; and tylan, 10 μg/ml) had been decanted, leaving only a thin film over the cells. The inoculum was left for 3 h to enable RLO to infect the cells, before washing three times with sterile phosphate-buffered saline. A 10-ml amount of sterile culture medium (M199 with 2 mM L-glutamine, 10 mM sodium bicarbonate, and 1% fetal calf serum) containing 50 μg of sulfacetamide per ml was added to the flasks, which were then incubated at 35°C.

The growth of RLO was monitored by gently scraping a few cells from the bottom of the flask and staining with Giminez stain (6). A dense growth of RLO could be seen after 5 days. On day 6 the cells were detached from the tissue culture flask with 5% trypsin. The RLO density was determined by microscopy of stained preparations and adjusted with brain heart infusion broth to approximately 300,000 organisms per ml. To confirm the maintainance of virulence, 0.1 ml of this was inoculated i.p. into three more male Hartley guinea pigs which were monitored as above. Three milliliters of the RLO suspension was stored in 0.3-ml aliquots at −80°C for use in cattle inoculation experiments.

The remaining suspension was inoculated into a 1-liter glass tissue culture flask containing a confluent layer of Vero cells to regrow the RLO. On day 5 of this subinoculation, the cells were harvested and RLO were purified by a modified method of Weiss et al. (15). The resulting pellet of organisms was resuspended in 0.5 ml of K36 buffer (11) and stored at −80°C until needed for IFA testing.

Identification of isolates. *R. conorii* Simko, a strain originally isolated by Philip et al. (11), was obtained from The National Institute for Virology, Republic of South Africa. Groups of five laboratory-bred BALB/c mice were inoculated i.p. with 0.05 ml of either the RLO suspension or *R. conorii* Simko and maintained separately in a tick-free envi-

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ronment. After 14 days, blood was collected by cardiac puncture and the pooled sera were tested by IFA against the homologous and heterologous antigen.

Inoculation of cattle. Eight healthy cross-bred steers varying in age from 8 to 18 months and seronegative by IFA for antibodies to *R. conorii* were used. They were maintained in a tick-free enclosure and fed a commercial ration and high quality hay ad lib. The animals were inoculated intradermally over the triceps muscle of the left shoulder with 0.3 ml of the stored RLO suspension such that five animals (HD1 through HD5) received a total dose of approximately 100,000 organisms, while for the remaining three animals (LD1 through LD3) the suspension was diluted with brain heart infusion broth to give a total of approximately 3,000 organisms. As a control, 0.3 ml of an uninfected brain heart infusion broth Vero cell suspension was injected above the inoculation sites.

All steers were examined each day for the duration of the experiment for clinical manifestations of disease. On day 7, the inoculation sites were biopsied under local anesthesia and submitted in 10% buffered Formalin for histopathological examination. Blood was collected by jugular venipuncture for routine hematology and biochemistry screens. The serological response to infection was monitored by IFA tests, using goat anti-bovine IgG and IgM (Kirkegaard & Perry Laboratories).

To determine whether the steers became rickettsemic postinfection, 3 ml of whole blood was inoculated, immediately after collection, i.p. into seronegative male Hartley guinea pigs. Body temperature was determined daily for 10 days, and each animal was inspected for scrotal swelling. They were bled at 10 to 14 days postinoculation for IFA testing.

RESULTS

Cattle serosurvey. The overall prevalence of antibodies to spotted fever group rickettsias in cattle from the south of the country was 90% (18 of 20 in Mbizi, 17 of 20 in Tanganda Halt, and 12 of 12 in Bulawayo). In the north of the country the seroprevalence was only 26% (11 of 40 in Harare, 3 of 20 in Copper Queen, 2 of 20 in Mugunje, 1 of 20 in Chakonda, and 14 of 20 in Murewa).

Rickettsial identification. Injection i.p. of the tick suspension into a susceptible guinea pig caused a febrile reaction (>39.5°C) on postinfection days (PID) 4 and 5. Scrotal swelling and edema were noted on PID 5 and persisted until day 9. Serum collected from the guinea pig before inoculation gave a negative IFA result (titer, <1/40), while serum collected PID 14 showed seroconversion with a titer to the RLO antigen of 1/640. Febrile reactions, scrotal swelling, and seroconversion (titers of >1/320) were noted in each of the guinea pigs inoculated with tissue culture RLO.

The serum of the mouse group infected with *R. conorii* Simko showed a titer of 1/512 against both Simko antigen and the local isolate antigen, while that of the mouse group infected with the local isolate showed titers of 1/256 and 1/512, respectively.

Clinical pathology in cattle. The steers that were artificially infected with the RLO remained healthy and continued to gain weight for the duration of the experiment. Clinical examinations revealed no abnormalities apart from regional lymphadenopathy beginning on PID 4 and continuing until day 14. A 1-cm erythematous, edematous, and tender plaque at the site of inoculation persisted from PID 3 to 12. No lesion was detectable at the control injection site. Aspirates of the lymph node stained with Giminez stain failed to reveal any RLO, but cytology of the smears showed a high proportion of large, pleomorphic lymphocytes consistent with reactive lymphadenopathy. Hematology and biochemistry results were within normal limits for steers of this age.

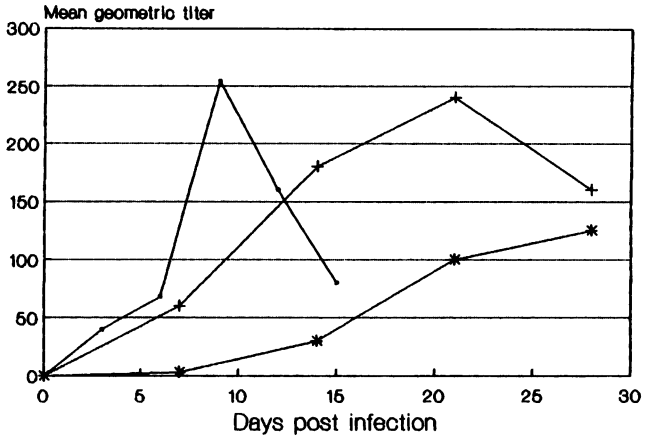

FIG. 1. Antibody responses to *R. conorii* after inoculation of cattle. Symbols: ●, mean LD IgM response; +, mean HD IgG response; *, mean LD IgG response. LD, Cattle inoculated with 3,000 organisms; HD, cattle inoculated with 100,000 organisms.

Histopathological examination of skin biopsies taken from the site of inoculation revealed a mild-to-moderate, acute-to-subacute deep dermatitis and vasculitis characterized by lymphocytic and plasmacytic infiltration.

Serlogy of experimentally infected cattle. Seroconversion was noted in all eight steers. Antibodies (titer, ≥1/40) appeared by PID 3 and persisted for at least 28 days (Fig. 1). The three animals inoculated with 3,000 organisms showed an IgM response first detectable at PID 3 and with maximum titers on day 9. All of the animals, whether inoculated with 3,000 or 100,000 organisms, showed an IgG response by PID 15, although animals inoculated with the higher dose usually showed an IgG response by PID 7.

Guinea pig inoculation. None of the 31 guinea pigs inoculated with blood from the cattle showed a pyrexia. Antibodies to the RLO were, however, detected at a titer of ≥1/40 in 12 of 24 (50%) animals from which a serum sample could be obtained (Table 1). Seroconversion was detected in guinea pigs inoculated with blood from steers that had received either large (HD cattle) or small (LD cattle) numbers of RLO 3 to 32 days previously. A greater number of guinea pigs that had received blood from HD cattle seroconverted (67%) compared with animals inoculated with LD cattle blood (40%).

DISCUSSION

Our knowledge of the epidemiology of the rickettsioses in southern Africa is based mainly on studies carried out in the Republic of South Africa in the 1930s and 1940s (3, 4, 7). These studies suggested that rodents may be reservoir hosts for *R. conorii* and that infection may be transmitted to humans by their ticks (4). The role of dogs as reservoirs is less clear, and it has been suggested that these animals are only important as paratenic hosts bringing infected ticks into the human environment (3). As far as we are aware, there have been no studies on the epidemiology of tick-bite fevers in Zimbabwe, although human cases are reported to be

TABLE 1. Serological responses of guinea pigs inoculated with blood from cattle artificially infected with rickettsias

Steer	Reciprocal IFA titer against *R. conorii* antigen on given day post-steer infection on which guinea pig was inoculated						
	3	6	9	10	12	23	32
LD1[a]	80	Neg	80	ND[b]	Neg	80	80
LD2	Neg	Neg	Neg	ND	Neg	Neg	160
LD3	Neg	Neg	160	ND	ND	ND	ND
HD1[c]	ND			80			
HD2	40			Neg			
HD3	160			Neg			
HD4	Neg			≥160			
HD5	40			≥160			

[a] Cattle were infected with 3,000 RLO.
[b] ND, Guinea pig died before serology could be done.
[c] Cattle were infected with 10^5 RLO.

common in the south of the country (9). This region is characterized by extensive cattle farming operations and a high prevalence of *A. hebraeum*, while in the north of the country this tick is found only in scattered foci. In this study we present evidence that cattle and their ticks may be implicated in the epidemiology of tick-bite fever.

This evidence is based on the following observations. (i) Cattle in the south of the country showed a high prevalence of antibodies reactive with *R. conorii*, while in the north of the country the prevalence was low. Although only a small number of cattle could be sampled in each area, the results were generally consistent. Thus, between 85 and 100% of cattle in the south of Zimbabwe were seropositive, while in the north, with the exception of Murewa (70%), the seroprevalence was <30%. This corresponds with the described distribution of *A. hebraeum*, which is found extensively in the south, but only in scattered foci in the north.

(ii) RLO could be readily isolated from *A. hebraeum* ticks. These RLO caused a typical febrile and scrotal reaction in guinea pigs and, when inoculated into mice, stimulated antibodies that were indistinguishable from antibodies to a known strain of *R. conorii* by comparative IFA tests. We would emphasize that we could not in the present experiments unequivocally confirm the isolate to be *R. conorii*. However, in view of the characteristic fever and scrotal reaction in guinea pigs, the cross-reactivity of our isolate in IFA tests with *R. conorii*, and the fact that *R. conorii* is the only spotted fever group rickettsia thus far reliably reported from Africa, we regard, for the purposes of this report, our isolate to be a strain of *R. conorii*. All stages of *A. hebraeum* readily feed on cattle and the larval stage is often found on humans (9).

(iii) The artificial inoculation of *R. conorii* into cattle caused an infection that was detectable serologically. Both IgM and IgG antibodies could be demonstrated by IFA, and the IgG response was dose related. The antibody titer of the low-dose inoculation was still rising on PID 28, indicating that the response was to an actual infection rather than just to the presence of antigen.

The 50% infective doses for *R. conorii* in guinea pigs and mice have been established as about 50 and 1,500 organisms, respectively (10), but that for cattle is unknown. The number of organisms used to infect cattle in these experiments was arbitrarily chosen, but the evidence we have indicates that the 50% infective dose is <3,000 organisms. Cattle thus are highly susceptible to *R. conorii* infection.

(iv) Infection in cattle produced only mild clinical symptoms of regional lyphadenopathy and cutaneous erythema, edema, and tenderness at the inoculation site where there was histological evidence of dermatitis and vasculitis similar to dermal rickettsial lesions described before (2, 8). Hematological and biochemical results were all within normal limits for steers of this age. Despite the lack of any systemic clinical symptoms, rickettsemia was demonstrated by inoculation of blood into guinea pigs. It should be noted that not all cattle had a detectable level of rickettsemia on all sampling days. The finding that more of the HD cattle blood-inoculated guinea pigs seroconverted suggests that rickettsemia is dose related. Animals exposed to large numbers of rickettsias would therefore appear to have higher concentrations of circulating organisms or be rickettsemic more consistently or both.

It is known that the minimum infective dose for guinea pigs is about 50 organisms for seroconversion, but about 300 organisms are needed to cause a febrile reaction (10). Since none of the guinea pigs that seroconverted showed febrile reactions, we estimate that the maximum number of circulating rickettsias in these experiments was 17 to 100 organisms per ml.

In summary, we have obtained evidence that a high percentage of cattle in an area endemic for human tick-bite fever show antibodies reactive with *R. conorii*; that *A. hebraeum*, common cattle ticks in this area, carry in their hemolymph RLO serologically indistinguishable from *R. conorii*; that cattle can be infected subclinically with this RLO; and finally, that these cattle show an intermittent rickettsemia for at least 32 days postinfection. The importance of these findings, in both veterinary and human medicine, is under investigation.

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