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SHORT REPORT: PREVALENCE OF ANTIBODIES AGAINST SPOTTED FEVER, MURINE TYPHUS, AND Q FEVER RICKETTSIAE IN HUMANS LIVING IN ZAMBIA

TAMAKI OKABAYASHI, FUTOSHI HASEBE, KENNY L. SAMUI, AARON S. MWEENE, SHANKER G. PANDEY, TSUYOSHI YANASE, YASUKAZU MURAMATSU, HIROSHI UENO, AND CHIHARU MORITA

School of Veterinary Medicine, Rakuno-Gakuen University, Hokkaido, Japan; School of Virology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan; Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia

Abstract. The causative agents of rickettsial diseases (*Rickettsia conorii, R. typhi,* and *Coxiella burnetii*) have been reported throughout the African continent. However, there have been no reports on epidemiologic surveys of these infections in Zambia. This study was designed to clarify the prevalence of three rickettsioses in 377 humans in Zambia. The seroprevalence of antibodies against *R. conorii, R. typhi,* and *C. burnetii* was 16.7%, 5.0%, and 8.2%, respectively. The rates of antibody positivity against *R. conorii* and *C. burnetii* were higher in the eastern (23.1% and 11.8%) and western (16.8% and 7.4%) areas of Zambia than in the northern (3.0% and 3.0%) area of this country. There was little difference among the three areas in the distribution of antibodies against *R. typhi.* Since cattle breeding is more extensive in the western and eastern areas than in the northern area, it is thought that cattle-breeding areas are foci of *R. conorii* and *C. burnetii* infections in Zambia.

Recently, serologic surveys of antibodies against spotted fever group (SFG) rickettsia, *Rickettsia typhi,* and *Coxiella burnetii* in humans living in African countries (Angola, Burkina Faso, Central African Republic, Comoros, Congo, Ivory Coast, Mali, and Tanzania) using an indirect immunofluorescent antibody (IFA) test have demonstrated a high prevalence of antibodies to rickettsiae.[1,2] However, there has been no seroepidemiologic survey of antibodies against rickettsiae in humans living in Zambia. In this study, the extent of infection with three rickettsioses (SFG rickettsia, murine typhus, and Q fever) in humans living in Zambia was investigated.

Serum samples were collected from inhabitants of Kasama (northern area), Chipata (eastern area), and Limulunga and Senanga (western area) Zambia by one of the authors (FH) in 1989 for an epidemiologic survey of Rift Valley fever.[3] These sera were collected after informed consent was obtained for surveys of infectious diseases. The study was approved by the School of Veterinary Medicine of the University of Zambia and the Graduate School of Veterinary Medicine of Rakuno-Gakuen University. Information on sex, age, occupation, contact with livestock (cattle), participation in meat processing, and handling of aborted fetuses of cattle was obtained by questionnaires at the time of the collection of blood samples. Sera were absorbed in blood sampling papers and dried according to the manufacturer's instructions for blood sampling filter paper (Toyo-Roshi, Tokyo, Japan). The papers were kept at 4°C until 1996 and eluted into 600 μl of phosphate-buffered saline (PBS), pH 7.5 (Nissui Pharmaceutical Co., Tokyo, Japan). The eluted serum was considered a 16-fold dilution.

The *R. conorii* Moroccan strain, the *R. typhi* Wilmington strain, and the *C. burnetii* Nine Mile phase II strain (VR-616; American Type Culture Collection, Rockville, MD) were grown and maintained in Vero E6 cells, BSC-40 cells, and BGM cells, respectively, and used as antigens of SFG rickettsia, murine typhus, and Q fever, respectively. *Rickettsia conorii* and *R. typhi* were provided by Dr. I. Kaiho (Department of Virology, Chiba Prefectual Public Health Laboratory, Chiba, Japan). The IFA test was performed as described by Morita and others.[4] Sera were serially diluted 2-fold with PBS. The antibodies were detected in fluorescein isothiocyanate–conjugated goat anti-human Ig A + IgM + IgG (Organon Teknika Corp., Durham, NC). To determine the lowest antibody-positive titers against *R. conorii, R. typhi,* and *C. burnetii,* we investigated the distribution of antibody titers against these agents in human serum samples analyzed by the IFA test. The distribution was biphasic, and showed a minimum at a dilution of 1:64. Serum titers ≥ 1:64 against the three agents were taken to be positive in this study. Antibody-positive sera against SFG rickettsia and *R. typhi* (provided by Dr. I. Kaiho) and antibody-positive sera against *C. burnetii* collected in Hokkaido, Japan were used as positive controls. Antibody-negative human sera against the three agents obtained from healthy people in Japan were used as negative controls.

The rates of antibody positivity against SFG rickettsia, *R. typhi,* and *C. burnetii* in the three areas of Zambia were 16.7%, 5.0%, and 8.2%, respectively (Table 1). Antibodies against SFG rickettsia were found more frequently in serum samples than those against *R. typhi* and *C. burnetii.* The prevalence of antibodies against SFG rickettsia and *C. burnetii* in the western and eastern areas of Zambia was significantly higher than that in the northern area ($P < 0.01$ and $P < 0.05$, respectively). The highest rate of antibody positivity against the three rickettsial disease agents was found in Chipata district. There were no significant differences among the three areas in the distribution of antibodies against *R. typhi.*

The prevalence of antibodies against the three rickettsial disease agents by age groups among humans sampled in this survey is shown in Table 1. Although the positivity rates of antibodies to *R. typhi* and *C. burnetii* were not related to age, the prevalence of positive sera against SFG rickettsia tended to increase with age. The rate of antibody positivity against SFG rickettsia in people ≥ 40 years old (24 of 95, 25.3%) was significantly higher than that in people less than 40 years old (33 of 256, 12.9%; $P < 0.01$; Table 2).

The relationships between the rickettsial antibody positive rate and the surveyed items (sex, age, occupation, involvement with cattle, participation in meat processing, and handling of aborted fetuses) are shown in Table 2. There were

TABLE 1

Prevalence of *Rickettsia conorii, R. typhi,* and *Coxiella burnetii* rickettsia infections in the study population

	Total	No. (%) of positive sera against		
		R. conorii	*R. typhi*	*C. burnetii*
District				
Kasama	101	3 (3.0)	6 (5.9)	3 (3.0)
Chipata	169	39 (23.1)*	10 (5.9)	20 (11.8)†
Limulunga Senanga	95	16 (16.8)*	3 (3.2)	7 (7.4)
Unknown	12	5 (41.6)	0	1 (8.3)
Total	377	63 (16.7)	19 (5.0)	31 (8.2)
Age (years)				
0–19	109	12 (11.0)	4 (3.7)	7 (6.4)
20–39	147	21 (14.3)	11 (7.5)	17 (11.6)
40–59	57	13 (22.8)	1 (1.8)	1 (1.8)
≥60	38	11 (29.0)	3 (7.9)	4 (10.5)

* $P < 0.01$.
† $P < 0.05$.

TABLE 2

Demographic information from individuals tested for antibodies against *Rickettsia conorii, R. typhi,* and *Coxiella burnetii*

Item	Total	No. (%) of positive sera against		
		R. conorii	*R. typhi*	*C. burnetii*
Sex				
Males	224	42 (18.8)	15 (6.7)	23 (10.3)
Females	141	16 (11.4)	4 (2.8)	7 (5.0)
Age				
<40 years	256	33 (12.9)	15 (5.9)	24 (9.4)
≥40 years	95	24 (25.3)*	4 (4.2)	5 (5.3)
Occupation				
Farming	114	20 (17.5)	5 (4.4)	13 (11.4)
Other	251	38 (15.1)	14 (5.6)	17 (6.8)
Meat processing				
Involvement	138	29 (21.0)	10 (7.2)	14 (10.1)
No involvement	239	34 (14.2)	9 (3.8)	17 (7.1)
Aborted fetuses				
Contact	87	17 (19.5)	5 (5.7)	10 (11.5)
No contact	290	46 (15.9)	14 (4.8)	21 (7.2)
Cattle				
Contact	137	28 (20.4)	9 (6.6)	14 (10.2)
No contact	209	27 (12.9)	8 (3.8)	16 (7.7)

* $P < 0.05$.

no significant differences in rates of antibody positivity related to any of the items of surveyed information.

Figure 1 shows the relationship between the rates of antibody positivity for the three rickettsia antibodies and the distribution of dairy farms (cattle-breeding areas) in Zambia.[5] These results indicate that these rickettsiae are widely spread in Zambia. Although there was no relationship between the rates of antibody positivity and the number of cattle handled, the prevalence of positive sera against SFG rickettsia and *C. burnetii* in two cattle-breeding areas (II and III) was higher than that in a nonbreeding area (I).

The epidemiologic results suggest that the seroprevalence of antibodies to SFG rickettsia coincides with the distribution (location) of cattle-breeding areas, and that the proportion of people with antibodies to SFG rickettsia increases with age. It was reported that positive sera against *R. conorii* reacted with equal intensity against *R. africae* in an IFA test,[1] but we could not distinguish between *R. conorii* and *R. africae* in this survey. High infestations of ticks on Zambian dairy farms have been reported[6] (Ngulube ET, University of Zambia, unpublished data). Recent data indicate that seroprevalence of antibodies reactive with SFG rickettsia is influenced by the distribution of ticks.[7] These results suggest that pastures in these areas of Zambia are inhabited by ticks, one of the main vectors of SFG rickettsia for humans.

The prevalence of antibodies against *C. burnetii* in people living in cattle-breeding areas and in people who are in close contact with cattle was high. The main route of *C. burnetii*

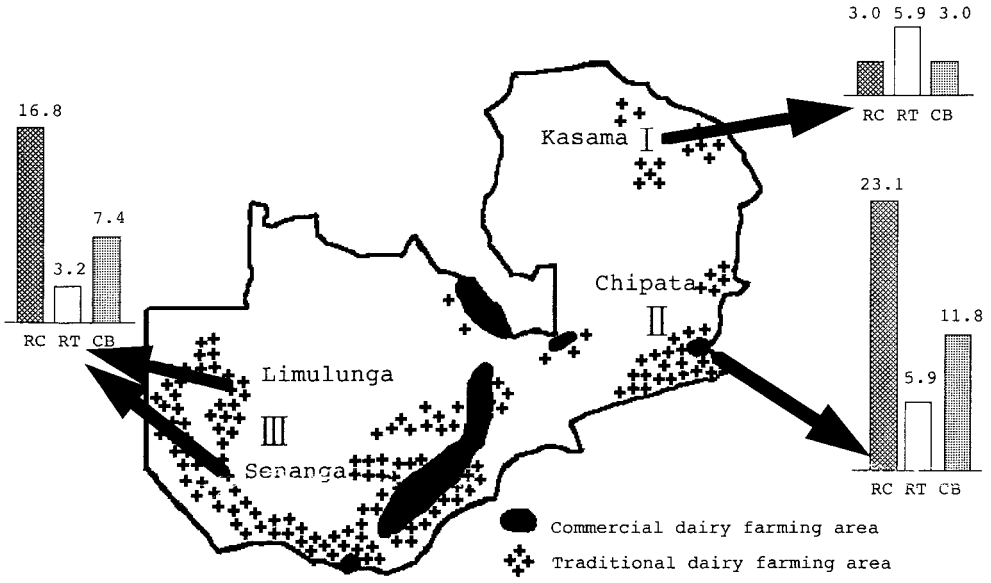

FIGURE 1. Map of Zambia showing prevalence (%) of antibodies against *Rickettsia conorii* (RC), *R. typhi* (RT), and *Coxiella burnetti* (CB) and distribution of cattle.

infection is through inhalation of contaminated aerosol containing the microorganism shed from infected animals.[8] The findings in this study may indicate that the presence of domestic animals is one of the risk factors for infection with this organism.

In the study of levels of antibodies against *R. typhi*, differences based on the information acquired from individuals were not found. The low rate of murine typhus in farming areas in this survey may be due to the fact that murine typhus is a disease of mostly urban or port areas.[2,9,10]

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Authors' addresses: Tamaki Okabayashi, Tsuyoshi Yanase, Yasukazu Muramatsu, Hiroshi Ueno, and Chiharu Morita, School of Veterinary Medicine, Rakuno-Gakuen University, Bunkyoudai Midori-machi 582, Ebetsu, Hokkaido, 069, Japan. Futoshi Hasebe, School of Virology, Institute of Tropical Medicine, Nagasaki University, 1–12–4 Sakamoto, Nagasaki, 852, Japan. Kenny L. Samui, Aaron S. Mweene, and Girja S. Pandey, Department of Disease Control, School of Veterinary Medicine, University of Zambia, PO Box 32379, Lusaka, Zambia.

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