Am. J. Trop. Med. Hyg., 95(2), 2016, pp. 462–465
doi:10.4269/ajtmh.15-0775

Evidence of *Rickettsia* and *Orientia* Infections among Abattoir Workers in Djibouti

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Abstract. Of 49 workers at a Djiboutian abattoir, eight (16%, 95% confidence interval [CI]: 9–29) were seropositive against spotted fever group rickettsiae (SFGR), two (4%, 95% CI: 1–14) against typhus group rickettsiae, and three (6%, 95% CI: 2–17) against orientiae. One worker (9%, 95% CI: 2–38) seroconverted against orientiae during the study period. This is the first evidence of orientiae exposure in the Horn of Africa. SFGR were also identified by polymerase chain reaction in 32 of 189 (11%, 95% CI: 8–15) tick pools from 26 of 72 (36%) cattle. Twenty-five (8%, 95% CI: 6–12) tick pools were positive for *Rickettsia africae*, the causative agent of African tick-bite fever. Health-care providers in Djibouti should be aware of the possibility of rickettsiae infections among patients, although further research is needed to determine the impact of these infections in the country.

Rickettsiae, obligate intracellular Gram-negative bacteria primarily transmitted to vertebrate hosts by arthropod vectors, are divided into four groups, two of which were examined in this study: spotted fever group rickettsiae (SFGR) and typhus group rickettsiae (TGR).[1] The closely related *Orientia* spp. causes scrub typhus, a disease similar to rickettsioses.[2] Although rickettsiae are found globally, orientiae are mainly endemic in the Asia–Australia–Pacific region, with limited evidence of infections in Africa,[3] the Middle East,[4] and South America.[5] As part of a broader study examining exposure to vector-borne and zoonotic pathogens in a high-risk environment in Djibouti, human samples were analyzed for evidence of exposure to and infection with rickettsiae and orientiae, and tick samples were examined for infection with rickettsiae.

Before study initiation, the research protocol was reviewed by the institutional review boards (IRBs) and Institutional Animal Care and Use Committees at U.S. Naval Medical Research Unit No. 3 (NAMRU-3, IRB protocol no. 911) and the U.S. Centers for Disease Control and Prevention (IRB protocol no. 5901), and by the Ministry of Health (MOH) and the Ministry of Agriculture, Livestock and the Sea in Djibouti. Samples were then collected at the Abattoir Frigorifique de Djibouti.

In September 2010, participating abattoir workers completed baseline questionnaires and provided blood samples. Every 4 weeks, for a total of 20 weeks, an additional blood sample and questionnaire were collected from workers who self-reported a recent history of acute febrile illness (AFI); no subsequent samples were collected from workers who did not report recent AFI. Serum was stored at −20°C at MOH, transferred to NAMRU-3 in Cairo, Egypt, on dry ice, and stored at −40°C. Aliquots of 49 baseline and 11 follow-up samples were sent to the Naval Medical Research Center (NMRC) in Silver Spring, Maryland, on dry ice. Serum samples were screened for IgG antibodies against whole-cell antigens of SFGR, TGR, and orientiae by enzyme-linked immunosorbent assays (ELISAs), as described previously.[6,7] An ELISA titer procedure was performed on all screen-positive samples. Western blot assays using the recombinant proteins Kpr56[6] and Kpr47b[7] and an immunofluorescence assay (IFA) (Fuller Laboratories, Fullerton, CA) using Karp, Kato, Gilliam, and Boryong whole-cell antigens were performed to confirm orientiae-ELISA positives. The proportion of workers with IgG antibodies against SFGR, TGR, and orientiae were calculated with Wilson confidence intervals (CIs).

Every 4 weeks during the study, ticks were collected from a convenience sample of freshly slaughtered cattle. On average, 190 (range: 118–303) ticks were collected from an average of 44 (range: 30–73) cattle at each sampling. Ticks were stored in plastic vials at −70°C at MOH, and were transferred to NAMRU-3 on dry ice. After taxonomic identification, ticks were pooled (1–3 ticks) by species, sex, and source animal. Total nucleic acid was extracted following procedures described elsewhere.[8] Aliquots of nucleic acid preparations were stored at −70°C, and a subset of 189 tick pools containing 306 ticks from 72 cattle imported from Ethiopia were sent to NMRC on dry ice. Tick nucleic acid preparations were tested for *Rickettsia* DNA using a *Rickettsia* genus–specific quantitative real-time polymerase chain reaction (qPCR) assay (Rick17b)[9]; samples positive by Rick17b were further tested by a qPCR assay specific for *Rickettsia africae* (including *R. africae* variants) (RafriG).[10] PCR, nested PCR, and sequencing of a fragment of *ompB* were carried out to confirm the identities of the *Rickettsia* spp.[9] Infection rates were calculated using maximum likelihood estimates, and are reported per 100 ticks with skewness-corrected score CIs.[11]

At baseline, eight (16%, 95% CI: 9–29) workers were seropositive for IgG antibodies against SFGR (geometric mean titer: 476, range: 100–1,600), two (4%, 95% CI: 1–14) for TGR (geometric mean titer: 200, range: 100–400), and two (4%, 95% CI: 1–14) for orientiae (geometric mean titer: 200, range: 100–400). Analysis of paired sera for 11 workers showed one (9%, 95% CI: 2–38) seroconversion for antibodies against orientiae during the study period. No seroconversions for SFGR or TGR were observed. Samples positive by orientiae ELISA were confirmed by IFA and the western blot assays against Kpr56 and/or Kpr47b (Table 1). Demographic and occupational characteristics of abattoir workers are shown in Table 2.

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TABLE 1

Results of abattoir workers' sera for orientiae IgG–specific antibodies by ELISA, IFA, and western blot assays

Participant ID	Time of sample collection	ELISA titer	IFA (1:128)	Western blot (1:100)	
				Kpr47b	Kpr56
12	Baseline	< 100	−	−	−
12	Week 4	400	+	±	+
37	Baseline	400	+	±	+
58	Baseline	100	+	+	ND*
40†	Baseline	< 100	−	−	−

ELISA = enzyme-linked immunosorbent assay; IFA = immunofluorescence assay.
*Not done (ND) because the sample was already depleted.
†An ELISA-negative serum sample from an abattoir worker.

SFGR were identified in 32 of 189 (11%, 95% CI: 8–15) pools of ticks from 26 of 72 (36%) cattle, of which 25 (8%, 95% CI: 6–12) were *R. africae*. Three types of *ompB* sequences that differed from *R. africae* were obtained: three (1%, 95% CI: 0–3) *R. africae* variant 1, two (1%, 95% CI: 0–2) *R. africae* variant 2, and two (1%, 95% CI: 0–2) *R. africae* variant 3; the sequence identity to *R. africae* was 99.4, 99.2, and 99.1%, respectively. *Rickettsia* sp. S strain was detected in two (1%, 95% CI: 0–2) pools, and unidentified *Rickettsia* spp. were detected in two (1%, 95% CI: 0–2) pools (Table 3). Six sequences were submitted to GenBank under accession numbers from KT032136 to KT032141.

Little is known about rickettsial disease in Djibouti. A 1995 study of Somali refugees in southern Djibouti showed serologic evidence of SFGR (e.g., *Rickettsia conorii*) and TGR (e.g., *Rickettsia typhi*) infection,[12] but to our knowledge, there is no existing documentation of rickettsial infection within the Djiboutian population. In the present study, 24% of abattoir workers showed evidence of prior infection with rickettsiae or orientiae, and one worker seroconverted against orientiae antigens during the study period, indicating possible ongoing transmission of orientiae in Djibouti. However, because the study population was limited to abattoir workers, it is not known whether these results reflect circulation beyond this high-risk environment. This report corroborates previous studies conducted in eastern Africa where prevalence of SFGR was found to be high and that of TGR low.[10,13]

This is, to our knowledge, the first evidence of orientiae exposure in the Horn of Africa. Although the traditional geographic range for scrub typhus does not include Africa, antibodies were reported in Rwanda and Burundi in the 1950s,[14] and individual visitors were likely infected in the Republic of Congo and Cameroon in the 1990s.[3,15] In addition, recent evidence of exposure to orientiae was reported among febrile patients in Kenya.[7] Travel history of participants in the present study is not known, so it is not possible to determine whether orientiae exposure occurred within Djibouti. However, based on the socioeconomic status of these three individuals, it is unlikely that any would have traveled outside the country.

The present study identified *R. africae*, the causative agent of African tick-bite fever, among 14 (16%, 95% CI: 9–25) pools of *Amblyomma lepidum*, 10 (63%, 95% CI: 37–86) pools of *Amblyomma variegatum*, and one (50%, 95% CI: 4–96) pool of *Rhipicephalus annulatus*. *Rickettsia africae* was first reported in Djibouti in 2007 in *A. lepidum* from cattle imported from Ethiopia, and has been identified in *A. lepidum* in Sudan as well.[16] Although not previously identified among *A. variegatum* in Djibouti, infection in this species is common throughout sub-Saharan Africa.[17] *Rickettsia africae* has also

TABLE 2

Demographic and occupational characteristics of abattoir workers with IgG antibodies against SFGR, TGR, or orientiae at any time during the study period

	SFGR		TGR		Orientiae		All participants	
	n	%	*n*	%	*n*	%	*n*	%
Nationality								
Djiboutian	8	100	2	100	2	67	46	94
Somali	0	0	0	0	1	33	2	4
Ethiopian	0	0	0	0	0	0	1	2
Sex								
Male	7	88	1	50	2	67	39	80
Female	1	13	1	50	1	33	10	20
Age								
< 30	0	0	0	0	0	0	3	6
30–39	1	13	1	50	1	33	17	35
40–49	4	50	1	50	2	67	15	31
≥ 50	3	38	0	0	0	0	14	29
Length of employment (years)*	27 (10–40)		14 (13–15)		17 (15–20)		18 (2–40)	
Occupational tasks								
Slaughter livestock	7	88	1	50	1	33	33	67
Skin carcasses	6	75	1	50	1	33	35	71
Cut carcasses and process organs	1	13	2	100	0	0	8	16
Clean abattoir facilities	1	13	0	0	1	33	6	12
Administrative work and/or security	0	0	0	0	2	67	3	6
Animal contact								
All animal species within the abattoir	3	37	0	0	2	67	17	35
Only cattle and/or camels within the abattoir	3	37	2	100	0	0	27	55
Only sheep and/or goats within the abattoir	2	25	0	0	0	0	5	10
Other animals outside abattoir	2	25	1	50	0	0	12	24
Total	8	100	2	100	3	100	49	100

SFGR = spotted fever group rickettsiae; TGR = typhus group rickettsiae.
*Average (minimum–maximum).

TABLE 3

Number of tick pools (1–3 ticks each) in which SFGR was detected by *Rickettsia* genus–specific qPCR, *Rickettsia africae*–specific qPCR, and *ompB* fragment sequencing with bias-corrected MLEs and skewness-corrected score CIs

Tick species	*Rickettsia*		*R. africae*		*R. africae* variant 1		*R. africae* variant 2		*R. africae* variant 3		*Rickettsia* sp. S strain		Unknown *Rickettsia* spp.		Total pools (total ticks)
	n	MLE (95% CI)	*n*	MLE (95% CI)	*n*	MLE (95% CI)	*n*	MLE (95% CI)	*n*	MLE (95% CI)	*n*	MLE (95% CI)	*n*	MLE (95% CI)	
Amblyomma cohaerens	0	0 (0–30)	0	0 (0–30)	0	0 (0–30)	0	0 (0–30)	0	0 (0–30)	0	0 (0–30)	0	0 (0–30)	6 (8)
Amblyomma lepidum	14	16 (9–25)	14	16 (9–25)	0	0 (0–4)	0	0 (0–4)	0	0 (0–4)	0	0 (0–4)	0	0 (0–4)	51 (99)
*Amblyomma variegatum**	13	90 (64–99)	10	63 (37–86)	3	16 (4–39)	2	10 (2–28)	2	9 (2–27)	0	0 (0–15)	0	0 (0–15)	14 (21)
Dermacentor spp.	0	0 (0–13)	0	0 (0–13)	0	0 (0–13)	0	0 (0–13)	0	0 (0–13)	0	0 (0–13)	0	0 (0–13)	18 (24)
Hyalomma marginatum	4	3 (1–6)	0	0 (0–3)	0	0 (0–3)	0	0 (0–3)	0	0 (0–3)	2	1 (0–4)	2	1 (0–4)	94 (148)
Rhipicephalus annulatus	1	50 (4–96)	1	50 (4–96)	0	0 (0–66)	0	0 (0–66)	0	0 (0–66)	0	0 (0–66)	0	0 (0–66)	2 (2)
Rhipicephalus spp.	0	0 (0–49)	0	0 (0–49)	0	0 (0–49)	0	0 (0–49)	0	0 (0–49)	0	0 (0–49)	0	0 (0–49)	4 (4)
Total	32	11 (8–15)	25	9 (6–12)	3	1 (0–3)	2	1 (0–2)	2	1 (0–2)	2	1 (0–2)	2	1 (0–2)	189 (306)

CI = confidence interval; MLE = maximum likelihood estimate; qPCR = quantitative real-time polymerase chain reaction; SFGR = spotted fever group rickettsiae.
*Three pools contained both *R. africae* and *R. africae* variant 1; one pool contained both *R. africae* and *R. africae* variant 2.

been previously found in *R. annulatus* in Guinea, Senegal, Nigeria, and Kenya,[17–19] but it is not clear whether this species of tick is a passive carrier of *R. africae* through cofeeding with infected ticks or feeding on an infected animal, or whether these ticks are able to maintain and transmit rickettsiae.

Three different genotypic variants of *R. africae* were detected in *A. variegatum* but not in *A. lepidum* as previously described.[16] The importance of these variants in disease transmission is currently unknown.

Rickettsia sp. S strain, a unique SFGR related to *R. africae*,[19] isolated in 1995 from *Rhipicephalus sanguineus* ticks collected from sheep in Armenia, was identified in two (2%) pools of *Hyalomma marginatum* ticks collected from a single cow of Ethiopian origin. The importance of this SFGR in human and veterinarian health is unknown at this time.

Further studies are needed to determine the extent and impact of rickettsial infections in Djibouti, and health-care providers should be aware of the possibility of these infections among their patients.

Received October 24, 2015. Accepted for publication April 15, 2016.

Published online June 6, 2016.

Acknowledgments: We acknowledge the management and workers from the abattoir for their cooperation and participation in the study. We also thank the MOH and NAMRU-3 personnel who conducted specimen collection.

Financial support: This project was funded by the Department of Defense Global Emerging Infections System (GEIS), work units 847705.82000.25GB.E0018 and 0000188M.0931.001.A0074, and the Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, U.S. Centers for Disease Control and Prevention.

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