Leptospirosis among Hospitalized Febrile Patients in Northern Tanzania

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Abstract. We enrolled consecutive febrile admissions to two hospitals in Moshi, Tanzania. Confirmed leptospirosis was defined as a ≥ 4 -fold increase in microscopic agglutination test (MAT) titer; probable leptospirosis as reciprocal MAT titer ≥ 800 ; and exposure to pathogenic leptospires as titer ≥ 100 . Among 870 patients enrolled in the study, 453 (52.1%) had paired sera available, and 40 (8.8%) of these met the definition for confirmed leptospirosis. Of 832 patients with ≥ 1 serum sample available, 30 (3.6%) had probable leptospirosis and an additional 277 (33.3%) had evidence of exposure to pathogenic leptospires. Among those with leptospirosis the most common clinical diagnoses were malaria in 31 (44.3%) and pneumonia in 18 (25.7%). Leptospirosis was associated with living in a rural area (odds ratio [OR] 3.4, $P < 0.001$). Among those with confirmed leptospirosis, the predominant reactive serogroups were Mini and Australis. Leptospirosis is a major yet underdiagnosed cause of febrile illness in northern Tanzania, where it appears to be endemic.

INTRODUCTION

Leptospirosis is a zoonotic disease that occurs in developed and developing countries worldwide. Leptospirosis is the result of infection with a spirochete, genus *Leptospira*, which is primarily transmitted by exposure to leptospire-contaminated urine from infected animals. Excreted leptospires may survive for days to months in freshwater, soil, or mud, and result in endemic and epidemic disease in both rural and urban settings.¹ In general, the incidence of leptospirosis tends to be underestimated and reflects the local availability of diagnostics and clinical index of suspicion.^{2,3}

The clinical manifestations of leptospirosis range from asymptomatic infection to severe, fatal illness, but leptospirosis most commonly presents as an undifferentiated febrile illness. Conventional diagnosis is by serology; the reference standard diagnostic test is the microscopic agglutination test (MAT). MAT requires advanced laboratory facilities and expertise, and is not widely available in low- and middle-income countries where leptospirosis may be endemic. Consequently, leptospirosis may be overlooked as a cause of febrile illness, and the burden of leptospirosis-associated morbidity and mortality remains poorly defined in many parts of the world. To assess the importance of leptospirosis as a cause of febrile illness in northern Tanzania, we evaluated hospitalized febrile patients for leptospirosis and described their characteristics.

MATERIALS AND METHODS

Setting. The study was conducted at two hospitals in Moshi, Tanzania. Moshi (population > 144,000) is the administrative center of the Kilimanjaro Region (population > 1.4 million) in northern Tanzania. It is situated at an elevation of ~890 meters above sea level, and the climate typically consists of

a short rainy period between October and December and a long rainy period from March to May.⁴ The Kilimanjaro Region is predominantly classified as rural with the exception of urban Moshi. Kilimanjaro Christian Medical Centre (KCMC) is a 458-bed consultant referral hospital that serves several regions in northern Tanzania, and Mawenzi Regional Hospital (MRH) is a 300-bed regional hospital that serves the Kilimanjaro Region. Together, KCMC and MRH are the main providers of hospital care in the Moshi area.

Study procedures and participants. As part of a comprehensive study of the etiology of febrile illness in northern Tanzania,^{5,6} we prospectively enrolled adult and pediatric inpatients at KCMC and MRH from 17 September 2007 through 31 August 2008. Adolescents and adults, defined as age ≥ 13 years and admitted to the adult medicine ward, were eligible to participate if they had an oral temperature of $\geq 38.0^{\circ}\text{C}$. Infants and children, defined as age ≥ 2 months to < 13 years and admitted to the pediatric ward, were eligible to participate if they had a history of fever in the past 48 hours, an axillary temperature of $\geq 37.5^{\circ}\text{C}$ or a rectal temperature of $\geq 38.0^{\circ}\text{C}$. A trained clinical officer who was part of the study team performed a standardized clinical history and physical examination on all consenting patients. Demographic information, including the participant's district and village of residence, was collected. Provisional clinical diagnoses provided by the hospital clinical team were recorded. Before administration of antimicrobial therapy and within 24 hours of hospital admission, blood was drawn for aerobic blood culture, mycobacterial blood culture, complete blood count, examination for blood parasites, human immunodeficiency virus (HIV) antibody and RNA testing,^{7–9} and acute serum archiving. At the time of discharge, a standardized form was completed documenting whether the patient died in hospital, in-hospital management, and the discharge diagnoses. The results of all available study investigations were provided immediately to the hospital clinical team to inform patient management. All participants were asked to return 4–6 weeks after study enrollment to submit a convalescent serum sample. Acute and convalescent serum samples were sent to the United States Centers for Disease Control and Prevention (CDC) for serologic analysis for leptospirosis.

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Laboratory methods. Leptospirosis laboratory diagnosis was made using the standard MAT performed at the CDC.¹⁰ Live leptospiral cell suspensions representing 20 serovars and 17 serogroups were incubated with serially diluted serum specimens. Resulting agglutination titers were read using darkfield microscopy. The reported titer was the highest dilution of serum that agglutinated at least 50% of the cells for each serovar tested.¹⁰

The serogroups (serovars) included in the antigen panel were Australis (*Leptospira interrogans* serovar Australis, *L. interrogans* serovar Bratislava), Autumnalis (*L. interrogans* serovar Autumnalis), Ballum (*Leptospira borgpetersenii* serovar Ballum), Bataviae (*L. interrogans* serovar Bataviae), Canicola (*L. interrogans* serovar Canicola), Celledoni (*Leptospira weilii* serovar Celledoni), Cynopteri (*Leptospira kirschneri* serovar Cynopteri), Djasiman (*L. interrogans* serovar Djasiman), Grippotyphosa (*L. kirschneri* serovar Grippotyphosa), Hebdomadis (*Leptospira santarosai* serovar Borincana), Icterohemorrhagiae (*L. interrogans* serovar Mankarso, *L. interrogans* Icterohemorrhagiae), Javanica (*L. borgpetersenii* serovar Javanica), Mini (*L. santarosai* serovar Georgia), Pomona (*L. interrogans* serovar Pomona), Pyrogenes (*L. interrogans* serovar Pyrogenes, *L. santarosai* serovar Alexi), Sejroe (*L. interrogans* serovar Wolffi), and Tarassovi (*L. borgpetersenii* serovar Tarassovi).

Study definitions. Confirmed leptospirosis was defined as a ≥ 4 -fold rise in the agglutination titer between acute and convalescent serum samples.¹¹ Probable leptospirosis was defined as a single reciprocal MAT titer ≥ 800 .^{3,12,13} Exposure to pathogenic leptospires was defined as any reciprocal MAT titer ≥ 100 .^{3,12} Predominant reactive serogroup was defined as the serogroup for the reacting serovar with the highest MAT titer. Rural or urban residence, based on the 2002 Tanzania Population and Housing Census, was determined on a village level for all those with complete residence demographics available.¹⁴

Statistical analysis. Data were entered using the Cardiff Teleform system (Cardiff, Inc., Vista, CA) into an Access database (Microsoft Corp., Redmond, WA). Descriptive statistics are presented as proportions, medians, ranges and interquartile ranges (IQR). Pearson's χ^2 was used to compare categorical data; Fisher's exact test was used when any cell contained fewer than 10 observations. Wilcoxon rank sum was used to compare categorical and nonparametric continuous data. Odds ratios (OR) and 95% confidence intervals (CI) were calculated when appropriate. For comparisons between those with confirmed leptospirosis and the rest of the study population, only those with paired sera available were compared. For comparisons between those with probable leptospirosis and the rest of the study population, only those with at least one serum sample available were compared. For analysis involving laboratory values, locally established reference ranges were used.^{15,16} Rainfall data from a weather station in the Kilimanjaro Region were used to determine the rainiest months (Noel HP, personal communication). All *P* values are 2-sided and evaluated for statistical significance at the 0.05 significance level. All analyses were performed using STATA, version 10.0 (Stata Corp., College Station, TX).

Research ethics. This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating

Committee, and Institutional Review Boards of Duke University Medical Center and the CDC.

RESULTS

A total of 870 patients with acute febrile illness were enrolled, 403 (46.3%) adults and adolescents and 467 (53.7%) infants and children. Serum was available for leptospirosis serology for 831 (95.5%); 453 (54.5%) participants had both acute and convalescent (paired) sera and 378 (45.5%) participants had a single serum sample (acute or convalescent). Median time between collection of acute and convalescent samples was 33 (IQR 31, 54) days. Forty (8.8%) of 453 patients with paired sera met the definition of confirmed leptospirosis. Of those with a single serum sample, 30 (3.6%) of 831 met the definition of probable leptospirosis. In all, 70 (8.4%) of 831 participants met the definition either confirmed or probable leptospirosis. Evidence of exposure to pathogenic leptospires was found in 277 (36.4%) of the 761 participants who had one or more titers available and did not meet criteria for confirmed or probable leptospirosis. In all, 346 (41.6%) of 831 participants had evidence of antibodies reacting to pathogenic leptospires.

The overall median (range) age of participants with serum available for leptospirosis serology was 10.3 (2.0, 95.8) years: 37.3 (13.6, 95.8) years among adolescents and adults and 1.5 (0.2, 13.5) years among infants and children. The overall median age among participants with confirmed or probable leptospirosis was 23.3 (0.3, 78.0) years: 39.8 (14.4, 78.0) years among adults and adolescents and 3.1 (0.3, 13.5) years among infants and children (Table 1). Participants with confirmed or probable leptospirosis were older than those without leptospirosis ($P < 0.001$). The median age of those with evidence of exposure to pathogenic leptospires was 23.8 (0.2, 88.7) years, whereas the median age for those without evidence of exposure to pathogenic leptospires was 4.5 (0.2, 95.8) years. The proportion of those with evidence of exposure to pathogenic leptospires increased as age increased ($P < 0.001$). There were 396 (47.7%) females in the overall study population and 18 (45.0%) of 40 patients with confirmed leptospirosis were female.

Clinical and laboratory characteristics. Clinical and laboratory characteristics are presented in Table 1. Median duration of illness before admission among participants with confirmed leptospirosis was 7 (IQR 4, 14) days for the adults and adolescents and 3 (IQR 2, 14) days for infants and children. The most common presenting symptoms were rigors, headache, and cough; one patient with confirmed leptospirosis had jaundice. No clinical symptoms distinguished those participants with confirmed or probable leptospirosis from the rest of the study population (P values > 0.05). Among adults and adolescents, no physical exam findings differed in those with confirmed or probable leptospirosis as compared with those without leptospirosis (P values > 0.05). Infants and children with confirmed or probable leptospirosis were more likely to have lymphadenopathy (25.9%) than those without leptospirosis (9.2%), ($P = 0.014$).

Twelve (57.1%) of 21 adult and adolescent patients with confirmed leptospirosis had thrombocytopenia. Thrombocytopenia was associated with confirmed (OR 3.5, $P = 0.005$) and probable (OR 2.2, $P = 0.017$) leptospirosis in adults and adolescents but not in infants and children ($P = 0.584$). Fourteen

TABLE 1
Characteristics of participants with and without leptospirosis, northern Tanzania, 2007–2008*

	Adults and adolescents			Infants and children		
	Confirmed† N = 21	Probable† N = 22	Neither† N = 352	Confirmed† N = 19	Probable† N = 8	Neither† N = 409
Female sex	12 (57)	12 (55)	189 (54)	6 (32)	1 (13)	176 (43)
Age median years (range)	42 (14, 71)	35 (15, 78)	36 (14, 96)	2.3 (0.4, 13)	7.6 (0.6, 11)	1.4 (0.2, 13)
Clinical symptoms and signs						
Fever	21 (100)	22 (100)	353 (100)	19 (100)	8 (100)	409 (100)
Temperature on admission °C (IQR)	39 (38, 39)	39 (38, 39)	39 (38, 39)	38 (38–39)	39 (38, 40)	38 (38, 39)
Days since onset of illness (range)	7 (4, 14)	11 (4, 30)	7 (3, 14)	3 (2–14)	4 (3, 7)	4 (3, 8)
Rigors	17 (81)	18 (82)	250 (71)	–	–	–
Headache	14 (67)	13 (59)	253 (72)	–	–	–
Cough	12 (57)	11 (50)	233 (66)	11 (58)	5 (63)	255 (63)
Vomiting	7 (33)	9 (41)	124 (35)	4 (21)	2 (25)	97 (24)
Breathing difficulty	6 (29)	10 (45)	117 (34)	9 (47)	2 (25)	154 (38)
Diarrhea	3 (14)	3 (14)	58 (17)	3 (16)	1 (13)	108 (26)
Photophobia	2 (10)	3 (14)	37 (11)	–	–	–
Hemoptysis	1 (5)	0 (0)	20 (6)	–	–	–
Neck stiffness	1 (5)	1 (5)	27 (8)	1 (5)	1 (13)	23 (6)
Jaundice	1 (5)	1 (5)	5 (1)	0 (0)	1 (13)	2 (< 1)
Hepatomegaly	2 (10)	4 (18)	27 (8)	4 (21)	2 (25)	40 (10)
Lymphadenopathy	1 (5)	5 (23)	34 (10)	7 (37)	0	37 (9)
Laboratory findings‡						
Leukocytosis	5 (24)	10 (45)	154 (44)	10 (53)	7 (88)	142 (35)
Leukopenia	3 (14)	3 (14)	30 (9)	0	0	21 (5)
Lymphopenia	14 (67)	8 (36)	167 (49)	2 (12)	3 (38)	87 (22)
Thrombocytopenia	12 (57)	8 (36)	100 (29)	1 (6)	1 (13)	22 (6)
Anemia	9 (43)	14 (64)	186 (53)	3 (18)	2 (25)	86 (22)
Chest radiograph findings (n)§	16	11	254	12	5	242
Normal	8 (50)	6 (55)	127 (50)	3 (25)	1 (20)	77 (32)
Infiltrates (including alveolar)	4 (25)	2 (18)	77 (30)	8 (67)	4 (80)	149 (62)
Pulmonary nodule (≥ 1)	2 (13)	0	16 (6)	1 (8)	0	11 (5)
Cavitary lesion	1 (6)	0	16 (6)	1 (8)	0	12 (5)
Pleural effusion	1 (6)	2 (18)	19 (7)	0	1 (20)	4 (2)
Treatment						
Antibacterial alone	1 (5)	9 (41)	131 (37)	11 (58)	2 (25)	230 (56)
Antimalarial alone	7 (33)	5 (23)	57 (16)	0	2 (25)	14 (3.4)
Antibacterial + antimalarial	11 (52)	8 (36)	121 (34)	5 (26)	2 (25)	87 (21)
No antibacterial or antimalarial documented	2 (10)	0	43 (12)	3 (16)	2 (25)	78 (19)
Provisional diagnosis						
Malaria	13 (62)	9 (41)	156 (44)	5 (26)	4 (50)	121 (30)
Pneumonia	3 (14)	6 (27)	90 (26)	7 (37)	2 (25)	141 (34)
Hepatitis	1 (5)	0	0	0	0	0
Meningitis	0	1 (5)	22 (6)	0	1 (13)	29 (7)
Other	4 (19)	5 (23)	85 (24)	7 (37)	1 (13)	118 (29)
Deaths before discharge	0	5 (23)	36 (10)	0	0	34 (8)

* Data are n (%) of patients unless otherwise indicated; IQR = interquartile range.

† Data not available for all patients for all variables, % reflects the accurate denominator.

‡ Laboratory reference ranges based on locally established reference ranges.^{15,16}

§ Radiographs may have multiple findings.

(66.7%) adults and adolescents with confirmed leptospirosis had lymphopenia. After controlling for HIV infection status, lymphopenia was not more common among those with confirmed leptospirosis compared with the rest of the study population ($P = 0.053$).

Diagnosis and treatment. Among those participants subsequently found to have confirmed or probable leptospirosis the most common provisional clinical diagnoses were malaria in 31 (44.3%), followed by pneumonia in 18 (25.7%). Clinical diagnosis of leptospirosis was not made in any case. Overall, 14 (20.0%) of those with confirmed or probable leptospirosis were treated with antimalarial drugs alone during admission, 23 (32.9%) were treated with antibacterial drugs alone, and 26 (37.1%) were treated with a combination of antimalarials and antibacterials. Of the 49 patients treated with antibacterial medications, 39 (79.6%) were treated with beta-lactams or chloramphenicol.

Residence demographics. Rural or urban residence could be determined for 713 (85.8%) participants; 356 (49.9%) resided in a rural area. Of 57 patients with residence data and confirmed or probable leptospirosis 43 (75.4%) lived in a rural area (OR 3.4, 95% CI 1.8–6.3, $P < 0.001$). There was no association between serologic evidence of exposure to pathogenic leptospires and rural residence (OR 1.3, 95% CI 1.0–1.8, $P = 0.091$). In the adult and adolescent study population, living in rural areas as compared with urban areas was associated with a lower level of education ($P < 0.001$), but there was no difference in monthly expenses, a surrogate of economic status ($P = 0.926$).

Prevalent serogroups. The predominant reactive serogroups for those participants with confirmed or probable leptospirosis are presented in Tables 2 and 3. The most commonly reacting serogroups among those with confirmed or probable leptospirosis were Mini and Australis. The serogroups to which

TABLE 2
Predominant infecting serogroup among those with confirmed leptospirosis, northern Tanzania, 2007–2008

Serogroup	Number positive N = 40	Acute titer	Convalescent titer	Median titer	Potential local reservoirs
Mini	13 (3*)	0	200–1600	200	Cattle, ¹⁷ hedgehogs, ¹⁸ dogs ¹⁹
Australis†	13	0–100	200–800	400	Rats, ^{20,21} cattle, ^{22,23} hedgehogs, ^{24,25} sheep, ^{22,26} dogs, ²⁷ donkeys ²²
Icterohaemorrhagiae‡	4 (3*)	0	200–800	200	Rats, ^{3,20,22} cattle, ^{17,22,23,28} dogs, ^{20,22,27,29} goats, ²² pigs, ¹⁷ donkeys ^{17,22}
Celledoni	3	0	200–800	800	Rodents ³⁰
Djasiman	1	0	400	400	Dogs ^{31,32}
Hebdomadis	1	0	200	200	Mice, ²⁰ goats, ²² sheep, ²² cattle, ^{17,22,23,33} donkeys, ¹⁷ chickens, ¹⁷ hippos ²²
Pyrogenes§	1	0	200	200	Rats, ²⁰ goats, ^{17,22} donkeys, ²² cattle, ^{21–23,29} pigs, ¹⁷ chickens ¹⁷
Tarassovi	1	0	200	200	Pigs, ^{17,34} cattle ^{23,35}

* Equal titers to serogroups Mini and Icterohaemorrhagiae.

† *L. interrogans* serovar Australis N = 3, *L. interrogans* serovar Bratislava N = 8, both *L. interrogans* serovar Australis and *L. interrogans* serovar Bratislava N = 3.

‡ *L. interrogans* serovar Icterohaemorrhagiae N = 4.

§ *L. interrogans* serovar Pyrogenes N = 1.

reactive titers ≥ 100 were found are presented in Table 4. The most prevalent serogroups in the population with antibodies to *Leptospira* spp. were Mini, Autumnalis, Australis, and Icterohaemorrhagiae.

Seasonal rainfall associations. No association was found between the rainiest months and cases with confirmed or probable leptospirosis ($P = 0.208$), including after accounting for days ill before admission and mean incubation period ($P = 0.829$).³⁶

Prevalence of co-infections. Among participants with confirmed leptospirosis, 16 (40.0%) had laboratory evidence of concurrent infection with one or more additional pathogens (Table 5). The most common concurrently positive results were spotted fever group rickettsiosis serology in 5 (12.5%), brucellosis serology in 5 (12.5%), and bacterial blood culture in 5 (12.5%). Other concurrently positive results included malaria microscopy in 3 (7.5%), Q fever serology in 1 (2.5%), and typhus group rickettsiosis serology in 1 (2.5%). Of the 16 patients with evidence of co-infection, 4 (25.0%) had evidence of two or more concurrent infections. Six (15.0%) patients with confirmed leptospirosis had HIV; there was no association between HIV and leptospirosis ($P = 0.177$). Evidence of co-infection was more common among adults and adolescents with confirmed leptospirosis than among infants and children; 12 (57.1%) adults and adolescents had evidence of co-infection as compared with 4 (21.0%) infants and children ($P = 0.027$).

Mortality. Five (7.1%) of 70 participants with evidence of leptospirosis died, all of whom were adults. Among those who died, 2 had HIV infection, 2 had documented diabetes and 1 had documented cirrhosis of the liver. Three (60.0%) of the 5 deaths occurred in those with bacterial bloodstream infections (2 with *Escherichia coli* and 1 with *Streptococcus pneumoniae*). Overall, those who died had either a bacterial bloodstream

infection or a clinically important co-morbidity in addition to probable leptospirosis; all were treated empirically with penicillin, ampicillin, or ceftriaxone.

DISCUSSION

Our study shows that leptospirosis is a common cause of febrile illness among inpatients in northern Tanzania. The high seroprevalence among patients without confirmed or probable leptospirosis, combined with the proportion of patients with confirmed or probable leptospirosis, suggests that the disease is endemic in northern Tanzania. Leptospirosis is difficult to distinguish clinically from other etiologies of febrile illness, such as malaria and bacteremia, which have received more attention in sub-Saharan Africa.³⁷ Furthermore, diagnostic tests for leptospirosis are not readily available and consequently the infection is rarely diagnosed by clinicians or managed with specific antimicrobial therapy.

The predominant reactive serogroups among participants with confirmed or probable leptospirosis were Mini and Australis. Among those with evidence of exposure to pathogenic leptospires, serogroups Mini, Australia, and Autumnalis were most prevalent. Although MAT is not a serovar-specific test, MAT may broadly indicate the common serogroups present in the local population^{38,39} and may in turn provide clues to important animal reservoirs. Serogroup Mini has not been previously well described in East Africa, but potential local reservoirs include cattle, dogs, and hedgehogs.^{17–19} Serogroup Australis has been described in cattle, sheep, rats, donkeys, dogs, and hedgehogs.^{20–27} Serogroup Autumnalis has been described in cattle, rats, mice, sheep, pigs, goats, and dogs.^{3,17,20,22} On the basis of the most common reactive serogroups in the study population, likely local reservoirs include livestock,

TABLE 3
Predominant infecting serogroup among those with probable leptospirosis, northern Tanzania, 2007–2008

Serogroup	Number positive N = 30	Single titer	Median titer	Potential local reservoirs
Mini	13	800–51,200	800	Cattle, ¹⁷ hedgehogs, ¹⁸ dogs ¹⁹
Australis*	8 (1†)	800–12,800	800	Rats, ^{20,21} cattle, ^{22,23} hedgehogs, ^{24,25} sheep, ^{22,26} dogs, ²⁷ donkeys ²²
Autumnalis	4 (1‡)	800–1,600	800	Rats, ²⁰ mice, ²⁰ cattle, ^{17,22} pigs, ¹⁷ sheep, ¹⁷ goats, ¹⁷ dogs, ³ hippos ²²
Grippotyphosa	1 (1†)	800–12,800	6,800	Cattle, ^{17,20–23} field mice, ²⁰ goats, ²⁰ pigs, ¹⁷
Canicola	1	1,600	1,600	Dogs, ²⁰ donkeys, ^{17,20}
Celledoni	1	1,600	1,600	Rodents ³⁰
Djasiman	1‡	800	800	Dogs ^{31,32}

* *L. interrogans* serovar Australis N = 1, *L. interrogans* serovar Bratislava N = 6, both *L. interrogans* serovar Australis and *L. interrogans* serovar Bratislava N = 1.

† Equal titers to serogroups Australis and Grippotyphosa.

‡ Equal titers to serogroups Autumnalis and Djasiman.

TABLE 4

Number of MAT titers ≥ 100 by serogroup, northern Tanzania, 2007–2008

Serogroup	Number of titers $\geq 100^*$
Mini	190
Autumnalis	121
Australis	101
Icterohaemorrhagiae	69
Celledoni	20
Djasiman	17
Canicola	7
Pyrogenes	6
Bataviae	5
Grippotyphosa	5
Tarassovi	5
Hebdomadis	5
Cynopteri	4
Sejroe	4
Ballum	2
Javanica	2
Pomona	1

*Any titer ≥ 100 within specified serogroup, all patients with sera available included ($N = 832$).

rodents, dogs, and potentially hedgehogs. Further studies are needed in northern Tanzania to define animal reservoirs and human risk factors for disease. These may include epidemiologic studies of persons with and without leptospirosis and animal studies to isolate and identify *Leptospira* in urine.

Although our study was not designed to comprehensively examine risk factors for leptospirosis in northern Tanzania, consistent with other studies we showed that residence in a rural area is associated with increased risk for leptospirosis.⁴⁰ The importance of livestock-based agriculture and pastoralism in rural Tanzania,^{4,41} and the predominance of reactivity to livestock-associated serovars identified by MAT underscore the possibility of an important role of livestock in leptospirosis transmission. Furthermore, persons living in rural areas in Tanzania are more likely to have exposure to surface water,⁴ a recognized risk factor for leptospirosis in other studies.⁴² In contrast with some studies,⁴³ we did not find a relationship between the rainy season and prevalence of acute leptospirosis. This may be because the available rainfall data are for

one location in the region, whereas the various districts may have actually experienced varied rainfall patterns and totals. Another possibility is that the exposure risk in this environment does not change substantially based on the season.

A substantial proportion of patients with confirmed leptospirosis in this study also had evidence of infection with another pathogen, most commonly spotted fever group rickettsiosis, brucellosis, or bacterial bloodstream infection. Possible explanations for these observations include true co-infection, cross-reactivity of the MAT, cross-reactivity of other serological tests (spotted fever group rickettsioses, *Brucella*) in the setting of leptospirosis, non-specific polyclonal immunoreactivity, or laboratory error. Although MAT specificity has been estimated at 96–98%,⁴⁴ cross-reactivity in the setting of other infections has been demonstrated.^{1,44} Alternatively, other studies also using conventional standard diagnostic tests, including identification of *Leptospira*, *Rickettsia*, and *Brucella* species by culture and molecular methods, have found evidence of simultaneous infections with more than one pathogen.^{45–47} In these studies, similar to ours, most patients with evidence of co-infection lived or worked in rural, agricultural settings.^{45,46} Although our study is unable to determine whether apparent co-infections represent true simultaneous infection or immunologic cross-reactivity, the environmental exposures experienced by persons living in northern Tanzania provide increased risk for several etiologic agents of febrile illness.

The clinical presentation of leptospirosis in this study was non-specific making clinical diagnosis challenging. Thrombocytopenia was the only laboratory marker associated with leptospirosis in adolescents and adults. In infants and children, no laboratory marker was specific for leptospirosis. This, combined with the focus on empiric treatment algorithms for febrile illness on malaria and sepsis,^{48,49} mean that leptospirosis is rarely diagnosed or treated with specific therapy in northern Tanzania. The consequences for patients of underdiagnosis of leptospirosis are difficult to measure. Because a confirmed diagnosis of leptospirosis requires acute and convalescent serum to be tested, estimating case fatality rate for patients with leptospirosis is difficult. For this reason, no patient with confirmed leptospirosis in our study died. Among those with an acute MAT titer of ≥ 800 , 5 (16.7%) died. However, all deaths were among adult patients with co-morbidities, making it difficult to attribute death to leptospirosis in these cases. A diagnostic test for leptospirosis with high sensitivity and specificity on an acute specimen would greatly assist with directing the antimicrobial management of patients and would provide a means to more accurately estimate the case fatality rate. Real-time polymerase chain reaction (PCR) is a promising method to assist with diagnosis in the acute leptospiremic phase,^{50,51} and should be further studied in Africa where local circulating *Leptospira* isolates need to be determined to optimize PCR performance.⁵²

In summary, leptospirosis is an important cause of febrile illness in northern Tanzania, where it appears to be endemic. MAT reactivity is most common to serogroups Mini, Australis, and Autumnalis. The serogroup reactivity, combined with rural residence as a risk factor, may suggest that livestock are important reservoirs in this area. Leptospirosis was difficult to distinguish clinically from other causes of febrile illness in this study and was never diagnosed or specifically treated. Greater awareness of leptospirosis among clinicians and efforts to develop a rapid and reliable diagnostic test that could be

TABLE 5

Concurrent infections among participants with serologic evidence of leptospirosis, northern Tanzania, 2007–2008*

Co-infection	Confirmed $N = 40$	Probable $N = 30$
Spotted fever group rickettsiosis†	5 (13)	1 (3)
Brucellosis†	5 (13)	2 (7)
Q fever†	1 (3)	1 (3)
Typhus group rickettsiosis†	1 (3)	0
Bacterial bloodstream infection	5 (13)	
<i>Streptococcus pneumoniae</i>	1 (3)	1 (3)
<i>Salmonella enterica</i> serotype Typhi	3 (8)	1 (3)
<i>Escherichia coli</i> and <i>Salmonella enterica</i> serotype Enteritidis	1 (3)	0
<i>E. coli</i>	0	2 (7)
Malaria‡	3 (8)	2 (7)
HIV	6 (15)	3 (10)
Overall number of patients with one or more co-infections, not including HIV	16 (40)	8 (27)
Patients with 2 or more co-infections, not including HIV	4 (10)	1 (3)

*Data are n (%) of patients unless otherwise indicated. HIV = human immunodeficiency virus.

†Diagnosed by serology.

‡Diagnosed by microscopy.

applied to acute specimens would improve patient management and facilitate estimation of the burden of morbidity and mortality. Further research is needed in northern Tanzania to define risk factors for human leptospirosis and to further define animal reservoirs to develop evidence-based prevention strategies.

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