Brucellosis among Hospitalized Febrile Patients in Northern Tanzania

Andrew J. Bouley, Holly M. Biggs, Robyn A. Stoddard, Anne B. Morrissey, John A. Bartlett, Isaac A. Afwamba,
Venance P. Maro, Grace D. Kinabo, Wilbrod Saganda, Sarah Cleaveland, and John A. Crump*

*Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham,
North Carolina; Bacterial Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, National Center for
Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Duke Global Health
Institute, Duke University, Durham, North Carolina; Kilimanjaro Christian Medical Centre, Moshi, Tanzania; Kilimanjaro Christian
Medical College, Tumaini University, Moshi, Tanzania; Mawenzi Regional Hospital, Moshi, Tanzania; College of Medicine,
Veterinary Medicine and Life Sciences, University of Glasgow, Glasgow, United Kingdom; Department of Pathology,
Duke University Medical Center, Durham, North Carolina*

Abstract. Acute and convalescent serum samples were collected from febrile inpatients identified at two hospitals in Moshi, Tanzania. Confirmed brucellosis was defined as a positive blood culture or a ≥ 4-fold increase in microagglutination test titer, and probable brucellosis was defined as a single reciprocal titer ≥ 160. Among 870 participants enrolled in the study, 455 (52.3%) had paired sera available. Of these, 16 (3.5%) met criteria for confirmed brucellosis. Of 830 participants with ≥ 1 serum sample, 4 (0.5%) met criteria for probable brucellosis. Brucellosis was associated with increased median age ($P = 0.024$), leukopenia (odds ratio [OR] 7.8, $P = 0.005$), thrombocytopenia (OR 3.9, $P = 0.018$), and evidence of other zoonoses (OR 3.2, $P = 0.026$). Brucellosis was never diagnosed clinically, and although all participants with brucellosis received antibacterials or antimalarials in the hospital, no participant received standard brucellosis treatment. Brucellosis is an underdiagnosed and untreated cause of febrile disease among hospitalized adult and pediatric patients in northern Tanzania.

INTRODUCTION

Brucellosis is an important cause of zoonotic illness worldwide.[1] Brucellosis frequently presents as an undifferentiated febrile illness with otherwise varied and non-specific clinical findings.[2–4] *Brucella* spp. are facultative intracellular organisms that can establish chronic infection, and brucellosis may present as acute, subacute, or chronic disease.[2,5] These non-specific clinical features, in addition to low clinical suspicion and inadequate diagnostic capacity, result in brucellosis often being underdiagnosed and untreated in low-resource countries.[3,6,7]

Although several species of *Brucella* can cause human infection, *B. melitensis* and *B. abortus* are the most frequently implicated species.[8,9] *B. melitensis*, the more common and virulent cause of human disease,[2,10] is typically associated with sheep and goats, and *B. abortus* with cattle, although cross-species infections can occur.[2,3,9] *Brucella* is transmitted to humans by ingestion of infectious animal products, inhalation of airborne particulates, or direct contact with infected animals and their products through skin abrasions or conjunctiva.[2] Although potentially more prevalent in northern Africa,[11,12] human brucellosis seroprevalence ranges from 3% to 8% in sub-Saharan Africa.[13–17] In livestock in Tanzania, the seroprevalence of *Brucella* infection varies across different agro-ecological settings, ranging from 0% to 15%.[18–20] The relative importance of the various *Brucella* species is not well-defined in sub-Saharan Africa. We previously isolated *B. melitensis* from the bloodstream of an inpatient in Moshi, Tanzania (Crump JA, unpublished data). *B. melitensis* has also been isolated from aborting goats and *B. abortus* from aborting cattle in Tanzania.[21] More recently, a single

B. melitensis isolate has been recovered from goat's milk in Tanzania (Shirima GM, unpublished data).

Brucellosis can negatively affect humans directly through illness, and economically by increasing the rate of abortion and decreasing productivity of livestock.[2,22] Livestock control measures that are effective in developed nations, including *Brucella* species-specific host vaccination and "test-and-slaughter" livestock culling strategies, have not been widely implemented at this time in sub-Saharan Africa.[23]

Although brucellosis awareness is increasing in sub-Saharan Africa,[9,23] few studies have systematically studied patients for evidence of infection using conventional standard diagnostic tests. To assess the role of brucellosis as a cause of febrile illness in northern Tanzania, and to add to the current understanding of human brucellosis in sub-Saharan Africa, we investigated the prevalence and characteristics of brucellosis among hospitalized febrile patients.

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.[24] With the exception of Moshi Urban District, the Kilimanjaro Region is predominantly classified as rural, and characterized by smallholder systems involving mixed crop and livestock farming. 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 serve as 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

---

*Address correspondence to John A. Crump, Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Box 102359, Durham, NC 27710. E-mail: john.crump@duke.edu

Tanzania,[25,26] we prospectively enrolled adult and pediatric inpatients at KCMC and MRH from 17 September 2007 through 31 August 2008. Adults and adolescents, ≥ 13 years of age, admitted to the adult wards were eligible to participate if they had an oral temperature of ≥ 38.0°C. Children and infants, aged ≥ 2 months to < 13 years, admitted to the pediatric wards were eligible to participate if they had a history of fever in the past 48 hours, an axillary temperature of ≥ 37.5°C, or a rectal temperature of ≥ 38.0°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 established 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, complete blood count, examination for blood parasites, human immunodeficiency virus (HIV) antibody testing,[27] and acute serum archiving. Infants and HIV antibody negative adults had HIV RNA testing performed.[28,29] Urine was also collected for detection of antibacterial activity. At the time of discharge, a standardized form was completed documenting whether the patient died in the 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 for collection of 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 brucellosis. More than 1 year after the final patient was enrolled serologic results were made available to the clinical team in Moshi, who attempted to trace the study participants with brucellosis for appropriate management.

Laboratory methods. Complete blood count and differential was performed using the CellDyn 3500 automated hematology analyzer (Abbott Laboratories, Abbott Park, IL). Blood cultures were performed using BacT/ALERT standard aerobic bottles, which were loaded into the BacT/ALERT 3D Microbial Detection system (BioMérieux Inc., Durham, NC) and were incubated for 5 days. Standard methods were used for identifying bloodstream isolates. Antimicrobial activity in urine was measured using a modification of the method described by Liu and others.[30]

Brucellosis serology was performed using the standard microagglutination test (MAT) performed at the CDC. Standardized *B. abortus* strain 1119-3 killed antigen (National Veterinary Services Laboratory, Ames, IA) was used for MAT at a 1:25 working dilution. Results were read on a Scienceware Plate Reader (Bel-Art Products, Wayne, NJ). Minor modifications were made to the CDC's standard MAT, including the use of U-bottom plates, incubation at 26°C, and discontinued use of staining techniques.[31]

Study definitions. Confirmed brucellosis was defined as a positive blood culture or a ≥ 4-fold rise in the total antibody titer between acute and convalescent serum samples. Probable brucellosis was defined as a single reciprocal titer ≥ 160.[31] For statistical analysis, negative for brucellosis was defined as any participant with paired serology who did not meet the criteria for confirmed or probable brucellosis. Nutritional status was assessed using sex-adjusted World Health Organiza-tion (WHO) guidelines for weight-for-length for children ≤ 24 months of age, weight-for-height for children > 24 and ≤ 60 months of age, and body mass index (BMI)-for-age for children and adolescents ≥ 61 months up to 19 years of age. Standard WHO adult BMI definitions were used for adults > 19 years of age.[32,33] Severely malnourished is defined as nutritional status < −3 SD by z-scores, and malnourished is defined as nutritional status < −2 SD. Hematologic definitions are based on locally established or verified reference ranges.[34,35] Rural or urban residence was based on the 2002 Tanzania Population and Housing Census for those with known village of origin.[36]

Statistical analysis. Data were entered using the Cardiff Teleform system (Cardiff Inc., Vista, CA) and analyzed using JMP, version 9.0.0 (SAS, Cary, NC). Descriptive statistics are presented as proportions, medians, ranges, and interquartile ranges (IQR). Fisher's exact test was used to compare categorical data. Wilcoxon rank sum was used to compare nonparametric continuous data. Odds ratios (ORs) and 95% confidence intervals were calculated when appropriate. For comparisons between those with confirmed brucellosis and the rest of the study population, only those with paired sera available were compared. All *P* values are two sided and evaluated for statistical significance at the 0.05 significance level. Probable brucellosis status was analyzed qualitatively.

Research ethics. Informed consent was obtained from all adult participants and from the parents or legal guardians of minors. This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institute 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 were enrolled in the study, 403 (46.3%) adults and adolescents and 467 (53.7%) children and infants. Characteristics of the study population have been reported elsewhere.[25,26] Eight hundred thirty (95.4%) participants had at least one serum sample available for brucellosis testing. Four hundred fifty-five (52.3%) enrollees had both acute and convalescent (paired) sera tested for brucellosis. Of the 455 participants with paired sera, 16 (3.5%) met criteria for confirmed brucellosis. No patient had a blood culture positive for *Brucella* spp.

Characteristics of those with and without brucellosis are presented in Table 1. The median (range) age of participants with paired sera for brucellosis analysis was 8.4 (0.2, 84.6) years: 1.8 (0.2, 13.5) years for children and infants and 34.5 (13.6, 84.6) years for adults and adolescents. The median (range) age of participants with confirmed brucellosis was 28.4 (1.1, 68.5) years: 2.1 (1.1, 3.6) years among children and infants and 46.0 (22.3, 68.5) years among adults and adolescents. Participants with confirmed brucellosis were significantly older than those without brucellosis (*P* = 0.024). Of those with paired sera tested for brucellosis, 223 (49.0%) were female, and 5 (31.3%) of the 16 patients with confirmed brucellosis were female.

Seven (43.8%) of the participants with brucellosis lived in a rural area. There was no association between brucellosis and rural residence (OR 0.85, *P* = 0.803). There was also no difference in weekly expenses for patients with and without brucellosis (*P* = 0.696).

TABLE 1
Presenting features of hospitalized febrile patients with and without brucellosis infection ($N = 454$), northern Tanzania, 2007–2008

	Confirmed brucellosis ($N = 16$)	Negative for brucellosis ($N = 438$)	P value	OR (95% CI)
	n/N (%)	n/N (%)		
Demographics				
Children and infants	5/16 (31.3)	243/438 (55.5)	0.073	0.36 (0.12–1.1)
Female sex	5/16 (31.3)	217/438 (49.5)	0.204	0.46 (0.16–1.4)
Age, median (range) years	28.4 (1.1, 68.5)	8.2 (0.2, 84.6)	0.024*	–
Adults and adolescents	46.0 (22.3, 68.5)	34.2 (13.6, 84.6)	0.154	–
Children and infants	2.1 (1.1, 3.6)	1.8 (0.2, 13.5)	0.784	–
Rural	7/16 (43.8)	183/382 (47.9)	0.803	0.85 (0.31–2.3)
Weekly expenses, median (IQR) TZS	20,000 (10,500; 28,000)	21,000 (14,000; 30,000)	0.696	–
Mortality	0/16 (0.0)	9/435 (2.1)	1.000	Undefined
Clinical symptoms and signs				
Days since onset, median (IQR)	7 (4, 14)	4 (3, 10)	0.224	–
Adults and adolescents	7 (4, 14)	6 (3, 14)	0.429	–
Children and infants	4 (3, 11)	4 (3, 7)	0.690	–
Temperature, median (IQR)	38.9 (38.2, 39.5)	38.4 (38.0, 39.0)	0.052	–
Headache†	8/11 (72.7)	145/194 (74.7)	1.000	0.90 (0.23–3.5)
Cough	5/16 (31.3)	287/436 (65.8)	0.007*	0.24 (0.08–0.69)
Vomiting	5/16 (31.3)	131/436 (30.0)	1.000	1.1 (0.36–3.1)
Breathing difficulty	3/16 (18.8)	154/438 (35.2)	0.284	0.43 (0.12–1.5)
Diarrhea	3/16 (18.8)	89/438 (20.3)	1.000	0.90 (0.25–3.2)
Jaundice	1/15 (6.7)	7/434 (1.6)	0.240	4.4 (0.50–37.9)
Pallor	0/16 (0.0)	35/437 (8.0)	0.625	Undefined
Crepitations	3/16 (18.8)	192/430 (44.7)	0.043*	0.29 (0.08–1.0)
Hepato- or splenomegaly	3/16 (18.8)	39/435 (9.0)	0.179	2.3 (0.64–8.6)
Lymphadenopathy	1/16 (6.3)	39/430 (9.1)	1.000	0.67 (0.09–5.2)
Severe malnutrition	0/15 (0.0)	52/418 (12.4)	0.234	Undefined
Any malnutrition	0/15 (0.0)	87/418 (20.8)	0.049*	Undefined
Laboratory findings				
Anemia	6/14 (42.9)	152/432 (35.2)	0.578	1.4 (0.47–4.1)
Lymphopenia	6/14 (42.9)	130/428 (30.4)	0.379	1.7 (0.58–5.1)
Thrombocytopenia	6/14 (42.9)	69/432 (16.0)	0.018*	3.9 (1.3–11.7)
Leukopenia	4/14 (28.6)	21/432 (4.9)	0.005*	7.8 (2.3–27.0)
Leukocytosis	4/14 (28.6)	160/432 (37.0)	0.587	0.68 (0.21–2.2)
HIV seropositive	1/15 (6.7)	107/418 (25.6)	0.130	0.21 (0.03–1.6)
Other bacterial zoonoses‡	7/16§ (43.8)	82/423 (19.4)	0.026*	3.2 (1.2–8.9)
Malaria	1/15 (6.7)	17/437 (3.9)	0.462	1.8 (0.22–14.2)
Bloodstream infections	1/16¶ (6.3)	45/438 (10.3)	1.000	0.58 (0.08–4.5)
Radiographic findings				
Abnormal chest radiograph	4/9 (44.4)	159/290 (54.8)	0.736	0.66 (0.17–2.5)
Infiltrates	3/9 (33.3)	128/290 (44.1)	0.736	0.63 (0.16–2.6)
Pleural effusion	1/9 (11.1)	11/290 (3.8)	0.312	3.2 (0.36–27.6)
Provisional diagnosis				
Malaria	8/16 (50.0)	200/438 (45.7)	0.801	1.2 (0.44–3.2)
Pneumonia	3/16 (18.8)	147/438 (33.6)	0.285	0.46 (0.13–1.6)
Meningitis	1/16 (6.3)	19/438 (4.3)	0.520	1.5 (0.18–11.7)
Other	4/16 (25.0)	72/438 (16.4)	0.322	1.7 (0.53–5.4)
Treatment				
Antibacterials pre-hospitalization	6/16 (37.5)	189/410 (46.1)	0.612	0.70 (0.25–2.0)
Antimalarials pre-hospitalization	10/16 (62.5)	159/415 (38.3)	0.067	2.7 (0.96–7.5)
Antibacterials in urine at enrollment	12/16 (75.0)	288/436 (66.1)	0.594	1.5 (0.49–4.9)
Antibacterials during hospitalization	13/15 (86.7)	301/438 (68.7)	0.165	3.0 (0.66–13.3)
Antimalarials during hospitalization	7/15 (46.7)	94/438 (21.5)	0.051	3.2 (1.1–9.1)
No antibacterials/antimalarials during hospitalization	0/15 (0.0)	111/438 (25.3)	0.028*	Undefined

* $P < 0.050$.
† Data available for adult and adolescent patients only.
‡ Includes confirmed cases of acute Q fever, leptospirosis, spotted fever group rickettsiosis (SFGR), typhus group rickettsiosis (TGR).
§ Serology positive for leptospirosis (4), SFGR (3), TGR (1), acute Q fever (1).
¶ Blood culture positive for *Salmonella enterica* serovar Typhi (1).
OR = odds ratio; CI = confidence interval; TZS = Tanzanian shilling; IQR = interquartile range.

Of the patients with brucellosis, 10 (62.5%) were Chagga by paternal tribe, 4 (25.0%) were Pare, and 1 (6.3%) was Wabondei. The paternal tribes for participants with and without brucellosis were not significantly different ($P = 0.067$).

Clinical, laboratory, and radiographic characteristics. The median (IQR) duration of illness before hospital admission was 7 (4,14) days for patients with brucellosis. Common symptoms among participants with brucellosis were subjective fever (100.0%), headache (72.7%), cough (31.3%), and vomiting (31.3%). Although cough was a common presenting symptom for participants with brucellosis, participants with brucellosis were less likely to have cough than febrile patients without brucellosis (OR 0.24, $P = 0.007$). Patients with brucellosis were less likely to have crepitations on chest auscultation (OR 0.29, $P = 0.043$), and were less likely to be malnourished (OR undefined, $P = 0.049$). No other symptoms or signs were

significantly different between participants with and without brucellosis (P values > 0.050).

Four (28.6%) participants with brucellosis had leukopenia, and 6 (42.9%) had thrombocytopenia, both significantly higher proportions than the participants without brucellosis (OR 7.8, P = 0.005 and OR 3.9, P = 0.018, respectively). There were no differences in chest radiograph findings between participants with and without brucellosis (P values > 0.050).

Prevalence of co-infections. One (6.7%) patient with confirmed brucellosis had HIV infection, and 107 (25.5%) participants without brucellosis had HIV (OR 0.21, P = 0.130). The patient co-infected with HIV and brucellosis had leukopenia, a CD4-positive T-lymphocyte count of 71 cells/mm^3, CD4% of 19.0, and was taking cotrimoxazole prophylaxis.

Seven (43.8%) participants with brucellosis had other confirmed bacterial zoonotic co-infections, including leptospirosis, spotted fever group rickettsiosis, typhus group rickettsiosis, and acute Q fever, a significantly higher proportion of other bacterial zoonotic infections than participants without brucellosis (OR 3.2, P = 0.026). One (6.7%) participant was co-infected with *Plasmodium falciparum*, and one (6.3%) participant was co-infected with *Salmonella enterica* serovar Typhi.

Diagnosis, treatment, and outcome. Of the participants with confirmed brucellosis, the most common provisional diagnosis was malaria in 8 (50.0%), followed by pneumonia in 3 (18.8%). Fifteen (93.8%) participants with brucellosis were diagnosed with malaria upon either admission or discharge. A clinical diagnosis of brucellosis was not made for any confirmed case. Clinical diagnoses for participants with brucellosis were not significantly different than those for patients with other febrile conditions (P > 0.050).

At the time of enrollment, 12 (75.0%) participants had evidence of urine antimicrobial activity. During admission, 13 (86.7%) of those with brucellosis received antibacterials and 7 (46.7%) received antimalarials, with 5 (33.3%) receiving both. All participants with brucellosis received either antibacterials or antimalarials; participants without brucellosis were more likely to have no treatment (P = 0.028). Of the 13 patients that received antibacterials during admission, 6 (46.2%) received penicillin or aminopenicillins, 4 (30.8%) received ceftriaxone, 3 (23.1%) received chloramphenicol, 2 (15.4%) received gentamicin, 2 (15.4%) received metronidazole, 1 (7.7%) received ciprofloxacin, and 1 (7.7%) received cotrimoxazole. None of the patients with confirmed brucellosis had a recorded death.

Probable cases. Of the 830 participants with any *Brucella* MAT result, 4 (0.5%) met criteria for probable brucellosis. Two of these four had residence information available, and both lived in a rural setting. Median (IQR) duration of illness before admission for those with probable brucellosis was 45 (9, 289) days. Two (50.0%) participants had hepato- or splenomegaly, 1 (25.0%) had lymphadenopathy, and 1 (33.3%) out of the 3 patients with BMI data was severely malnourished. On chest radiograph, 2 (66.6%) of the 3 patients with data had pleural effusions, and 1 (33.3%) had infiltrates. One (25.0%) participant was co-infected with HIV. Of the 4 participants with probable brucellosis, 1 (25.0%) died before discharge from the hospital. This patient had a 1-year history of recurrent fever and cough, two empiric treatments for tuberculosis without clinical improvement, and a large right-sided empyema.

DISCUSSION

We show that brucellosis is an underappreciated cause of febrile disease among adult and pediatric hospitalized patients in northern Tanzania. Although brucellosis is present in Tanzania, it was not diagnosed clinically in any study participant. Consequently, no participant received standard treatment of brucellosis during admission.[2] Increased clinician awareness and prompt access to reliable diagnostic tests is needed to identify and appropriately manage patients with brucellosis in northern Tanzania.

This study was not designed to comprehensively assess risk factors for brucellosis. However, it is notable that no association between brucellosis and rural residence, weekly expenditures, or paternal tribe was identified. Brucellosis has been well documented among nomadic pastoralists in sub-Saharan Africa,[16,22,37,38] and these populations are generally thought to be at increased risk for contracting brucellosis because of their close contact with livestock.[22] However, some studies have suggested a recent shift in the epidemiology of brucellosis from rural to urban settings, possibly related to unpasteurized milk consumption in urban environments.[39,40] A high *Brucella* seroprevalence among cattle in smallholder dairy systems in the Moshi area suggests that this sector may be a possible source of infection for the urban population.[18] With a larger sample size, an association between brucellosis and site of residence, socio-economic status, or tribe may have been observed.

The clinical presentation of brucellosis among our study participants was quite non-specific. Participants with brucellosis were significantly older than patients without brucellosis, suggesting that adult patients may be exposed to greater risk for the infection. Common reported clinical signs of human brucellosis include hepato- or splenomegaly and lymphadenopathy.[41–48] Our data showed no association between these signs and brucellosis. Although hepato- or splenomegaly was not significantly associated with brucellosis, possibly caused by small numbers, a larger proportion of individuals with brucellosis had hepato- or splenomegaly compared with those without brucellosis.

Consistent with other research, we found that thrombocytopenia and leukopenia were associated with brucellosis.[42,43] Although anemia was not associated with brucellosis in our study, it is notable that 42.9% of the participants with brucellosis with hematologic data had anemia. This proportion with anemia is comparable to that found in other studies.[41–43,49,50] Several studies have identified patients with pulmonary brucellosis and pleural effusions.[51–55] Although we identified 1 (11.1%) patient with brucellosis and pleural effusion, it was not a consistent finding in our cohort.

Available literature on the interaction between HIV and brucellosis is mixed. Two recent cross-sectional seroprevalence studies in Iran have shown an association between HIV and brucellosis seroprevalence.[56,57] Case reports have proposed an association between HIV and *B. canis*,[58,59] and between HIV and relapsing brucellosis.[60] Consistent with our study, others have found no association between HIV and brucellosis.[61,62] However, we did find that participants with brucellosis were more likely to have other bacterial zoonoses compared with participants without brucellosis. Although this may be caused by serologic cross-reactivity, prior studies have also shown co-infections with other zoonoses among

patients with brucellosis,[37,63] suggesting shared risk factors for these infections.

Our study eligibility criteria and case definitions were not optimized to detect chronic brucellosis. We defined confirmed acute brucellosis on the basis of a ≥ 4-fold increase in MAT titer between acute and convalescent serum samples. Those meeting the definition for probable brucellosis may have had chronic brucellosis, in which an increase in MAT titer would not be expected. Although no conclusions can be drawn because of sample size, it is interesting to note that the median duration of illness before enrollment of patients with probable brucellosis was 45 days. Others have shown extended delays in health-seeking behaviors among individuals with brucellosis in sub-Saharan Africa.[6] A large proportion of individuals with probable brucellosis also had several clinical signs classically ascribed to brucellosis, including hepato- or splenomegaly, lymphadenopathy, and radiographic evidence of pleural effusions. The one participant with probable brucellosis who did not survive to hospital discharge died with complications of a chronic pulmonary infection other than tuberculosis. Although we were unable to confirm the responsible pathogen, the clinical picture could be consistent with chronic brucellosis with pulmonary involvement.

Our study had several limitations. First, despite the relatively large sample size of the overall study, relatively few cases of confirmed brucellosis were identified. One consequence was that the sample size was too small to permit a multivariable analysis. Second, no confirmed case of brucellosis had a *Brucella*-positive blood culture. Prior antibacterial use may have lowered the sensitivity of the *Brucella* blood cultures, as 12 (75.0%) confirmed brucellosis cases had evidence of antibacterials in the urine at the time of enrollment, and 6 (37.5%) self-reported antibacterial use before hospitalization. Furthermore, in patients with serum agglutination test results suggestive of brucellosis, *Brucella*-positive blood culture prevalence is relatively low, ranging from 32% to 58%.[44,64–66] The third limitation is that our study catchment population may not have captured the full agro-ecologic diversity of northern Tanzania. In particular, nomadic pastoralists, a group at particular risk for brucellosis,[16,22,37,38] may have been underrepresented among KCMC or MRH inpatients. Finally, case definitions biased our study toward the detection of acute brucellosis, whereas those with chronic brucellosis may contribute greatly to the burden of illness and death. Furthermore, because a confirmed case required collection of convalescent serum 4–6 weeks after enrollment, it was impossible to identify acute brucellosis-associated deaths for participants with blood cultures negative for *Brucella*.

We show that brucellosis is an underappreciated and often misdiagnosed cause of febrile illness among hospitalized patients in sub-Saharan Africa. Simple, rapid diagnostic tests with adequate performance characteristics on acute specimens are needed to assist with the identification of patients with brucellosis.[8,67,68] To better prevent human brucellosis, it is important to target control of brucellosis in animal reservoirs,[3] including the use of the *Brucella* species-specific *B. melitensis* Rev 1 and *B. abortus* S19 and RB51 vaccines.[69] Closer collaboration between human and animal health experts is needed to better define which *Brucella* species are responsible for both human and animal brucellosis in sub-Saharan Africa. Additional research regarding local risk factors for human brucellosis, and the epidemiology of both human and animal brucellosis, is necessary to develop evidence-based prevention strategies in Tanzania.

Authors' addresses: Andrew J. Bouley, Holly M. Biggs, Anne B. Morrissey, John A. Bartlett, and John A. Crump, Department of Medicine, Duke University Medical Center, Durham, NC, E-mails: andrew.bouley@duke.edu, holly.biggs@duke.edu, amorr1023@gmail.com, john.bartlett@duke.edu, and john.crump@duke.edu. Robyn A. Stoddard, Bacterial Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: frd8@cdc.gov. Isaac A. Afwamba, Venance P. Maro, and Grace D. Kinabo, Kilimanjaro Christian Medical Centre, Moshi, Tanzania, E-mails: afwamba@gmail.com, venmaro@ymail.com, and gkinabo@hotmail.com. Wilbrod Saganda, Mawenzi Regional Hospital, Moshi, Tanzania. Sarah Cleaveland, College of Medicine, Veterinary Medicine and Life Sciences, University of Glasgow, Glasgow, United Kingdom, E-mail: sarah.cleaveland@glasgow.ac.uk.

REFERENCES

1. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV, 2006. The new global map of human brucellosis. *Lancet Infect Dis 6:* 91–99.
2. Corbel MJ, Elberg SS, Cosivi O, 2006. *Brucellosis in Humans and Animals*. Geneva: World Health Organization.

3. Godfroid J, Scholz HC, Barbier T, Nicolas C, Wattiau P, Fretin D, Whatmore AM, Cloeckaert A, Blasco JM, Moriyon I, Saegerman C, Muma JB, Al Dahouk S, Neubauer H, Letesson JJ, 2011. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Prev Vet Med 102:* 118–131.

4. Al Dahouk S, Nockler K, Hensel A, Tomaso H, Scholz HC, Hagen RM, Neubauer H, 2005. Human brucellosis in a non-endemic country: a report from Germany, 2002 and 2003. *Eur J Clin Microbiol Infect Dis 24:* 450–456.

5. Roop RM 2nd, Gaines JM, Anderson ES, Caswell CC, Martin DW, 2009. Survival of the fittest: how *Brucella* strains adapt to their intracellular niche in the host. *Med Microbiol Immunol (Berl) 198:* 221–238.

6. Kunda J, Fitzpatrick J, Kazwala R, French NP, Shirima G, Macmillan A, Kambarage D, Bronsvoort M, Cleaveland S, 2007. Health-seeking behavior of human brucellosis cases in rural Tanzania. *BMC Public Health 7:* 315.

7. John K, Kazwala R, Mfinanga GS, 2008. Knowledge of causes, clinical features and diagnosis of common zoonoses among medical practitioners in Tanzania. *BMC Infect Dis 8:* 162.

8. Franco MP, Mulder M, Gilman RH, Smits HL, 2007. Human brucellosis. *Lancet Infect Dis 7:* 775–786.

9. Pappas G, 2010. The changing *Brucella* ecology: novel reservoirs, new threats. *Int J Antimicrob Agents 36 (Suppl 1):* S8–S11.

10. Pappas G, Akritidis N, Bosilkovski M, Tsianos E, 2005. Brucellosis. *N Engl J Med 352:* 2325–2336.

11. Afifi S, Earhart K, Azab MA, Youssef FG, El Sakka H, Wasfy M, Mansour H, El Oun S, Rakha M, Mahoney F, 2005. Hospital-based surveillance for acute febrile illness in Egypt: a focus on community-acquired bloodstream infections. *Am J Trop Med Hyg 73:* 392–399.

12. Reddy EA, Shaw AV, Crump JA, 2010. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis 10:* 417–432.

13. John K, Fitzpatrick J, French N, Kazwala R, Kambarage D, Mfinanga GS, MacMillan A, Cleaveland S, 2010. Quantifying risk factors for human brucellosis in rural northern Tanzania. *PLoS ONE 5:* e9968.

14. Kassahun J, Yimer E, Geyid A, Abebe P, Newayeselassie B, Zewdie B, Beyene M, Bekele A, 2006. Sero-prevalence of brucellosis in occupationally exposed people in Addis Ababa, Ethiopia. *Ethiop Med J 44:* 245–252.

15. Swai ES, Schoonman L, 2009. Human brucellosis: seroprevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. *Zoonoses Public Health 56:* 183–187.

16. Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M, Zinsstag J, 2003. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Prev Vet Med 61:* 279–293.

17. Baba MM, Sarkindared SE, Brisibe F, 2001. Serological evidence of brucellosis among predisposed patients with pyrexia of unknown origin in the north eastern Nigeria. *Cent Eur J Public Health 9:* 158–161.

18. Swai ES, Mshanga D, Sanka NP, Marandu NH, 2005. Prevalence of bovine brucellosis in smallholder dairy farming area, Moshi, Tanzania. *Bull Anim Health Prod Afr 53:* 97–105.

19. Shirima GM, Cleaveland S, Kazwala RR, Kambarage DM, French N, McMillan A, Kunda J, Mfinanga GS, Fitzpatrick J, 2007. Sero-prevalence of brucellosis in smallholder dairy, agro-pastoral, pastoral, beef ranch and wildlife animals in Tanzania. *Bull Anim Health Prod Afr 55:* 13–21.

20. Karimuribo ED, Ngowi HA, Swai ES, Kambarage DM, 2007. Prevalence of brucellosis in crossbred and indigenous cattle in Tanzania. *Livestock Research for Rural Development 19:* 2007. Available at: http://www.lrrd.org/lrrd19/10/kari19148.htm. Accessed May 2, 2012.

21. Mahlau EA, 1967. Further brucellosis surveys in Tanzania. *Bull Epizoot Dis Afr 15:* 373–378.

22. McDermott JJ, Arimi SM, 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet Microbiol 90:* 111–134.

23. Marcotty T, Matthys F, Godfroid J, Rigouts L, Ameni G, Gey van Pittius N, Kazwala R, Muma J, van Helden P, Walravens K, de Klerk LM, Geoghegan C, Mbotha D, Otte M, Amenu K, Abu Samra N, Botha C, Ekron M, Jenkins A, Jori F, Kriek N, McCrindle C, Michel A, Morar D, Roger F, Thys E, van den Bossche P, 2009. Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcomes of a multi-disciplinary workshop. *Ann Trop Med Parasitol 103:* 401–411.

24. National Bureau of Statistics, ORC Macro, 2005. *Tanzania Demographic and Health Survey 2004–05.* Dar es Salaam, Tanzania: National Bureau of Statistics and Calverton, MD: ORC Macro, 381.

25. Crump JA, Ramadhani HO, Morrissey AB, Msuya LJ, Yang LY, Chow SC, Morpeth SC, Reyburn H, Njau BN, Shaw AV, Diefenthal HC, Bartlett JA, Shao JF, Schimana W, Cunningham CK, Kinabo GD, 2011. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected children and infants in northern Tanzania. *Trop Med Int Health 16:* 830–837.

26. Crump JA, Ramadhani HO, Morrissey AB, Saganda W, Mwako MS, Yang LY, Chow SC, Morpeth SC, Reyburn H, Njau BN, Shaw AV, Diefenthal HC, Shao JF, Bartlett JA, Maro VP, 2011. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania. *Clin Infect Dis 52:* 341–348.

27. Mayhood MK, Afwamba IA, Odhiambo CO, Ndanu E, Thielman NM, Morrissey AB, Shao JF, Pence BW, Crump JA, 2008. Validation, performance under field conditions, and cost-effectiveness of Capillus HIV-1/HIV-2 and Determine HIV-1/2 rapid human immunodeficiency virus antibody assays using sequential and parallel testing algorithms in Tanzania. *J Clin Microbiol 46:* 3946–3951.

28. Scott LE, Crump JA, Msuya E, Morrissey AB, Venter WF, Stevens WS, 2011. Abbott RealTi*me* HIV-1 *m2000rt* viral load testing: manual extraction versus the automated *m2000sp* extraction. *J Virol Methods 172:* 78–80.

29. Crump JA, Scott LE, Msuya E, Morrissey AB, Kimaro EE, Shao JF, Stevens WS, 2009. Evaluation of the Abbott *m*2000*rt* RealTi*me* HIV-1 assay with manual sample preparation compared with the ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR 1.5 using specimens from East Africa. *J Virol Methods 162:* 218–222.

30. Liu YC, Huang WK, Huang TS, Kunin CM, 1999. Detection of antimicrobial activity in urine for epidemiologic studies of antibiotic use. *J Clin Epidemiol 52:* 539–545.

31. Brown SL, Klein GC, McKinney FT, Jones WL, 1981. Safranin O-stained antigen microagglutination test for detection of *Brucella* antibodies. *J Clin Microbiol 13:* 398–400.

32. WHO Multicentre Growth Reference Study Group, 2006. *WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and Development.* Geneva: World Health Organization. Available at: http://www.who.int/childgrowth/standards/technical_report/en/index.html. Accessed January 10, 2012.

33. WHO Expert Committee on Physical Status, 1995. *Physical Status: The Use and Interpretation of Anthropometry.* Report of a WHO Expert Committee. Geneva: World Health Organization.

34. Buchanan AM, Muro FJ, Gratz J, Crump JA, Musyoka AM, Sichangi MW, Morrissey AB, M'Rimberia JK, Njau BN, Msuya LJ, Bartlett JA, Cunningham CK, 2010. Establishment of hematological and immunological reference values for healthy Tanzanian children in Kilimanjaro Region. *Trop Med Int Health 15:* 1011–1021.

35. Saathoff E, Schneider P, Kleinfeldt V, Geis S, Haule D, Maboko L, Samky E, de Souza M, Robb M, Hoelscher M, 2008. Laboratory reference values for healthy adults from southern Tanzania. *Trop Med Int Health 13:* 612–625.

36. Government of Tanzania, 2002. *2002 Population and Housing Census.* Dar es Salaam, Tanzania: Government of Tanzania.

37. Ari MD, Guracha A, Fadeel MA, Njuguna C, Njenga MK, Kalani R, Abdi H, Warfu O, Omballa V, Tetteh C, Breiman RF, Pimentel G, Feikin DR, 2011. Challenges of establishing the correct diagnosis of outbreaks of acute febrile illnesses in Africa: the case of a likely *Brucella* outbreak among nomadic

pastoralists, northeast Kenya, March–July 2005. *Am J Trop Med Hyg 85:* 909–912.

38. Omer MM, Musa MT, Bakhiet MR, Perrett L, 2010. Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. *Rev Sci Tech 29:* 663–669.

39. Makita K, Fevre EM, Waiswa C, Kaboyo W, De Clare Bronsvoort BM, Eisler MC, Welburn SC, 2008. Human brucellosis in urban and peri-urban areas of Kampala, Uganda. *Ann N Y Acad Sci 1149:* 309–311.

40. Dahouk SA, Neubauer H, Hensel A, Schoneberg I, Nockler K, Alpers K, Merzenich H, Stark K, Jansen A, 2007. Changing epidemiology of human brucellosis, Germany, 1962–2005. *Emerg Infect Dis 13:* 1895–1900.

41. Barroso Garcia P, Rodriguez-Contreras Pelayo R, Gil Extremera B, Maldonado Martin A, Guijarro Huertas G, Martin Salguero A, Parron Carreno T, 2002. Study of 1,595 brucellosis cases in the Almeria province (1972–1998) based on epidemiological data from disease reporting. *Rev Clin Esp 202:* 577–582.

42. Kokoglu OF, Hosoglu S, Geyik MF, Ayaz C, Akalin S, Buyukbese MA, Cetinkaya A, 2006. Clinical and laboratory features of brucellosis in two university hospitals in southeast Turkey. *Trop Doct 36:* 49–51.

43. Troy SB, Rickman LS, Davis CE, 2005. Brucellosis in San Diego: epidemiology and species-related differences in acute clinical presentations. *Medicine (Baltimore) 84:* 174–187.

44. Mantur BG, Biradar MS, Bidri RC, Mulimani MS, Veerappa, Kariholu P, Patil SB, Mangalgi SS, 2006. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. *J Med Microbiol 55:* 897–903.

45. Giannakopoulos I, Nikolakopoulou NM, Eliopoulou M, Ellina A, Kolonitsiou F, Papanastasiou DA, 2006. Presentation of childhood brucellosis in Western Greece. *Jpn J Infect Dis 59:* 160–163.

46. Andriopoulos P, Tsironi M, Deftereos S, Aessopos A, Assimakopoulos G, 2007. Acute brucellosis: presentation, diagnosis, and treatment of 144 cases. *Int J Infect Dis 11:* 52–57.

47. Buzgan T, Karahocagil MK, Irmak H, Baran AI, Karsen H, Evirgen O, Akdeniz H, 2010. Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. *Int J Infect Dis 14:* e469–e478.

48. Ariza J, Pigrau C, Canas C, Marron A, Martinez F, Almirante B, Corredoira JM, Casanova A, Fabregat J, Pahissa A, 2001. Current understanding and management of chronic hepatosplenic suppurative brucellosis. *Clin Infect Dis 32:* 1024–1033.

49. Hasanjani Roushan MR, Mohrez M, Smailnejad Gangi SM, Soleimani Amiri MJ, Hajiahmadi M, 2004. Epidemiological features and clinical manifestations in 469 adult patients with brucellosis in Babol, Northern Iran. *Epidemiol Infect 132:* 1109–1114.

50. Tsolia M, Drakonaki S, Messaritaki A, Farmakakis T, Kostaki M, Tsapra H, Karpathios T, 2002. Clinical features, complications and treatment outcome of childhood brucellosis in central Greece. *J Infect 44:* 257–262.

51. Simsek F, Yildirmak MT, Gedik H, Kanturk A, Iris EN, 2011. Pulmonary involvement of Brucellosis: a report of six cases. *Afr Health Sci 11 (Suppl 1):* S112–S116.

52. Kochar DK, Sharma BV, Gupta S, Jain R, Gauri LA, Srivastava T, 2003. Pulmonary manifestations in brucellosis: a report on seven cases from Bikaner (north-west India). *J Assoc Physicians India 51:* 33–36.

53. Hatipoglu CA, Bilgin G, Tulek N, Kosar U, 2005. Pulmonary involvement in brucellosis. *J Infect 51:* 116–119.

54. Pappas G, Bosilkovski M, Akritidis N, Mastora M, Krteva L, Tsianos E, 2003. Brucellosis and the respiratory system. *Clin Infect Dis 37:* e95–e99.

55. Kerem E, Diav O, Navon P, Branski D, 1994. Pleural fluid characteristics in pulmonary brucellosis. *Thorax 49:* 89–90.

56. Abdollahi A, Morteza A, Khalilzadeh O, Rasoulinejad M, 2010. Brucellosis serology in HIV-infected patients. *Int J Infect Dis 14:* e904–e906.

57. Hajiabdolbaghi M, Rasoulinejad M, Abdollahi A, Paydary K, Valiollahi P, SeyedAlinaghi S, Parsa M, Jafari S, 2011. *Brucella* infection in HIV infected patients. *Acta Med Iran 49:* 801–805.

58. Lawaczeck E, Toporek J, Cwikla J, Mathison BA, 2011. *Brucella canis* in a HIV-infected patient. *Zoonoses Public Health 58:* 150–152.

59. Lucero NE, Maldonado PI, Kaufman S, Escobar GI, Boeri E, Jacob NR, 2010. *Brucella canis* causing infection in an HIV-infected patient. *Vector Borne Zoonotic Dis 10:* 527–529.

60. Ibarra V, Blanco JR, Metola L, Oteo JA, 2003. Relapsing brucellosis in a patient infected with the human immunodeficiency virus (HIV). *Clin Microbiol Infect 9:* 1259–1260.

61. Moreno S, Ariza J, Espinosa FJ, Podzamczer D, Miro JM, Rivero A, Rodriguez-Zapata M, Arrizabalaga J, Mateos R, Herrero F, 1998. Brucellosis in patients infected with the human immunodeficiency virus. *Eur J Clin Microbiol Infect Dis 17:* 319–326.

62. Paul J, Gilks C, Batchelor B, Ojoo J, Amir M, Selkon JB, 1995. Serological responses to brucellosis in HIV-seropositive patients. *Trans R Soc Trop Med Hyg 89:* 228–230.

63. Parker TM, Murray CK, Richards AL, Samir A, Ismail T, Fadeel MA, Jiang J, Wasfy MO, Pimentel G, 2007. Concurrent infections in acute febrile illness patients in Egypt. *Am J Trop Med Hyg 77:* 390–392.

64. Al-Attas RA, Al-Khalifa M, Al-Qurashi AR, Badawy M, Al-Gualy N, 2000. Evaluation of PCR, culture and serology for the diagnosis of acute human brucellosis. *Ann Saudi Med 20:* 224–228.

65. Mantecon Mde L, Gutierrez MP, Zarzosa Mdel P, Fernandez-Lago L, Colmenero Jde D, Vizcaino N, Bratos MA, Almaraz A, Cubero A, Munoz MF, Rodriguez Torres A, Orduna A, 2008. Influence of brucellosis history on serological diagnosis and evolution of patients with acute brucellosis. *J Infect 57:* 397–403.

66. Young EJ, 1991. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. *Rev Infect Dis 13:* 359–372.

67. Lucero NE, Escobar GI, Ayala SM, Silva Paulo P, Nielsen K, 2003. Fluorescence polarization assay for diagnosis of human brucellosis. *J Med Microbiol 52:* 883–887.

68. Irmak H, Buzgan T, Evirgen O, Akdeniz H, Demiroz AP, Abdoel TH, Smits HL, 2004. Use of the *Brucella* IgM and IgG flow assays in the serodiagnosis of human brucellosis in an area endemic for brucellosis. *Am J Trop Med Hyg 70:* 688–694.

69. Schurig GG, Sriranganathan N, Corbel MJ, 2002. Brucellosis vaccines: past, present and future. *Vet Microbiol 90:* 479–496.