

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

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Abstract. An outbreak of acute febrile illness was reported among Somali pastoralists in remote, arid Northeast Kenya, where drinking raw milk is common. Blood specimens from 12 patients, collected mostly in the late convalescent phase, were tested for viral, bacterial, and parasitic pathogens. All were negative for viral and typhoid serology. Nine patients had *Brucella* antibodies present by at least one of the tests, four of whom had evidence suggestive of acute infection by the reference serologic microscopic agglutination test. Three patients were positive for leptospiral antibody by immunoglobulin M enzyme-linked immunosorbent assay, and two were positive for malaria. Although sensitive and specific point-of-care testing methods will improve diagnosis of acute febrile illness in developing countries, challenges of interpretation still remain when the outbreaks are remote, specimens collected too late, and positive results for multiple diseases are obtained. Better diagnostics and tools that can decipher overlapping signs and symptoms in such settings are needed.

INTRODUCTION

Diagnosing acute febrile illnesses (AFI) in much of Africa remains challenging for multiple reasons, including delayed recognition and reporting of outbreaks, the need to test for multiple potential pathogens, lack of adequate diagnostic facilities and methods in field laboratories, and inability to collect optimal specimen types (e.g., blood cultures, timely acute and convalescent sera collection). We investigated an outbreak of AFI in remote northeastern Kenya that highlights some of these challenges, as well as suggesting possible improvements in AFI diagnostics for such settings.

METHODS

Setting and case identification. On July 6, 2005, the Disease Outbreak Management Unit (now referred to as Division of Disease Surveillance and Response) of the Kenya Ministry of Health (now the Ministry of Public Health and Sanitation) received a report about an outbreak of AFI among six persons in an arid part of Northeastern Province, in Damajale sub-location (population 10,075, 1999 Census), 18 km from the Somali border and 250 km by dirt road from the district hospital. The community is predominantly ethnic Somali nomadic pastoralists. An outbreak of Chikungunya virus was originally suspected because of contemporaneous outbreaks along the Kenya coast characterized by fever and joint pains.¹ Therefore, a case definition compatible with the presentation of Chikungunya virus was used; any person living in Damajale sub-location who presented with new onset of fever or joint pains since March 1, 2005 (since the first cases of AFI in the area were reported in March). A field team was sent to

Damajale on July 18, 2005. Case-finding was undertaken by interviewing local health officials and community leaders and a review of medical records.

Laboratory testing. Blood was collected from suspected cases. Blood smears for malaria parasite and Widal tests were performed at the District Hospital and sera were transported in cool boxes to KEMRI-Centers for Disease Control and Prevention (CDC) International Emerging Infections Program laboratories in Nairobi. In Nairobi, serologic testing (immunoglobulin M [IgM] and IgG) were performed using enzyme-linked immunosorbent assay (ELISA) for the following pathogens; Chikungunya and O'nyong-nyong viruses, Yellow fever, West Nile, Rift Valley fever, and dengue viruses. Sera were also tested for leptospirosis using the Pan-Bio plate IgM ELISA kit (Panbio Limited, Brisbane, Australia). *Brucella* serologic testing was done using the Rose-Bengal test and complement fixation tests.² Frozen aliquots were sent later to the U.S. Naval Medical Research Unit-3 (NAMRU-3) laboratory in Cairo for tube agglutination² and rapid ELISA for *Brucella*, using a recently developed method in which a titer of > 1/320 was considered positive and ≥ 2 -fold higher IgM titer than IgG considered indicative of acute infection.³

Frozen sera were also sent to CDC laboratories in Atlanta. On the basis of results from the NAMRU-3 laboratory, testing was repeated using the reference serology method, the *Brucella* microagglutination test (BMAT), a modified format of standard tube agglutination test, which has been used for decades as a reference method for *Brucella* testing.⁴ Agglutination tests for *Brucella* detect antibodies of IgM, IgG, and IgA classes; to differentiate IgM from IgG this test is conducted in the presence (reduced test) and absence (unreduced test) of 2-Mercaptoethanol (2-ME).⁵ The 2-ME is a reducing agent that digests IgM and is therefore useful in distinguishing IgM from IgG activity and acute from chronic infections.^{5,6} A 4-fold difference in titer between the unreduced and reduced test of a single serum specimen is considered diagnostic of acute brucellosis.

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TABLE 1
Demographic and other information of patients from the acute febrile illness outbreak in Northeast Province Kenya, 2005*

ID	Age		Onset date	Specimen	Symptoms	Treatment
ID	Years	Sex	2005	Time post onset collected	At time of specimen collection	Took antibiotics
1	6	M	May 18	9 weeks	No	Yes
2	6	M	March 27	16 weeks	No	No
3	15	M	June 9	6 weeks	No	No
4	20	F	March 7	19 weeks	Yes	No
5	14	F	April 7	15 weeks	No	No
6	19	F	March 23	17 weeks	No	Yes
7	7	M	June 15	5 weeks	Yes	No
8	8	M	March 7	19 weeks	No	No
9	4	NA	July 6	2 weeks	Yes	No
10	3	NA	July 24	1 day	Yes	No
11	3	M	March 2	20 weeks	No	No
12	4	M	April 7	15 weeks	No	No

*NA = not available.

RESULTS

Twelve persons meeting the case definition were identified in Damajale. All case-patients had crossed the border into Somalia during the month before illness onset. Families of all respondents owned camels and cows from which they consumed unboiled milk. The community collected water from a single common borehole shared with livestock and stored it without treatment in narrow-mouthed plastic jerry cans.

Illness onset ranged from March to July 2005 (Table 1). Eight (62%) cases were under 10 years of age (range 2–20 years). Eight (62%) cases were male. The predominant symptoms were joint pain (100%), fever (75%), weight loss (58%), and headache (50%). No patients reported respiratory or gastrointestinal symptoms. At the time of the team's visit on July 18–25, 4 (33%) persons still had symptoms; the median number of days of symptoms for these four persons was 24.5 days (Table 1).

Nine of 12 sera showed evidence for acute or remote (i.e., previous infection at an unknown time) *Brucella* infection by at least one of the tests (Table 2). Of the 12 sera, Rose Bengal and complement fixation tests were positive in two and seven patients, respectively. The ELISA was positive in 8 of 10 tested for total Ig; 7 were positive by IgM, and 7 by IgG. Of the same 10 sera tested by BMAT, 5 were considered positive (≥ 160) including 4 with evidence suggestive of acute infection (4-fold

reduction in titer between unreduced and reduced test). Two patients still had symptoms and were within 2 weeks of onset; both demonstrated acute *Brucella* infection by BMAT. Of the other 2 still symptomatic patients, one did not have sufficient serum for testing and the other, who had sera drawn 19 weeks after symptom onset, was only positive by *Brucella* ELISA IgM, but not BMAT. All four patients with evidence suggestive of acute infection by BMAT had evidence of IgM by ELISA but one did not meet criteria for acute infection by ELISA (≥ 2 -fold higher IgM titer than IgG).

In addition to those patients positive for *Brucella*, three patients were IgM positive for leptospirosis, one of whom was also positive for acute brucellosis by BMAT (Table 2). Blood smears were positive for *Plasmodium falciparum* and *Plasmodium malariae* in one patient each; the *P. malariae* patient was also positive for acute *Brucella* by BMAT. Widal tests for typhoid and serologic testing for all viruses were negative.

DISCUSSION

This investigation of an outbreak in a remote part of Kenya highlights several of the challenges in diagnosing outbreaks of AFI in Africa. First, the outbreak was not identified until several months after the first cases occurred. Second, the outbreak site was in a remote location with difficult access. Both

TABLE 2
Results of serologic testing on patients from the acute febrile illness outbreak in Northeast Province Kenya, 2005*

ID	Brucella serology tests							Leptospira	
	Rose-Bengal test	CFT titer result/titer	SAT titer pos ≥ 160	ELISA total Ig titer pos ≥ 320	ELISA IgM titer pos ≥ 320	ELISA IgG titer pos ≥ 320	BMAT unreduced titer†	BMAT reduced titer	IgM ELISA
1	Borderline	Pos/4:20	Neg	640	640	160	160	40	Neg
2	Neg	Pos/4:20	Neg	1,280	320	320	160	160	Neg
3	Neg	Neg	Neg	160	160	320	40	< 20	Neg
4	Neg	Neg	Neg	320	320	320	≤ 20	< 20	Neg
5	Neg	Neg	ND	ND	ND	ND	ND	ND	Pos
6	Borderline	Pos/4:10	Neg	2,560	320	640	80	80	Neg
7‡	Borderline	Neg	ND	ND	ND	ND	ND	ND	Neg
8	Neg	Pos/4:20	160	640	320	160	640	80	Pos
9‡	Pos (weak)	Pos/4:40	160	1,280	1,280	320	640	160	Neg
10	Pos	Pos/4:10	640	5,120	5,120	320	2,560	640	Neg
11	Neg	Pos/3:40	Neg	320	160	320	40	40	Neg
12	Neg	Neg	Neg	160	160	< 160	< 20	< 20	Pos

*CFT = complement fixation test; SAT = standard tube agglutination test; ELISA = enzyme-linked immunosorbent assay; BMAT = *Brucella* microagglutination test; ND = not done.

†BMAT titers < 20 is negative, ≥ 160 with 4-fold reduction on the reduced test considered diagnostic of acute *Brucella* infection (values in bold font). No antibodies to chikungunya, West Nile, Yellow fever, and O'nyon-yong virus, and *Salmonella* (Widal test) detected.

‡Patients 7 and 9 were blood smear positive for *Plasmodium falciparum* and *Plasmodium malariae*, respectively.

of these factors led to collection of a limited number of specimens, collected mostly late in the course of illness when they were less useful diagnostically. The difficulties in accessing the area made collection of convalescent sera among symptomatic patients challenging. Third, lack of nearby adequate diagnostic facilities prohibited collection of some specimens that might have been the most useful, in this case blood cultures and convalescent-phase sera. Fourth, AFI is a nonspecific illness, caused by multiple pathogens in much of Africa for which distinguishing acute and remote infection is difficult. Besides brucellosis, patients in this outbreak tested positive for two types of malaria and leptospirosis, although it was not certain if these pathogens were causes of the acute illness. Last, the diagnostic tests currently available for many causes of AFI remain difficult to interpret.

Despite undergoing laboratory testing for several etiologies during the initial assessment of this outbreak of AFI, the role of the probable cause, *Brucella*, did not become clear until several months later after comprehensive testing in several reference laboratories. For diseases such as brucellosis or leptospirosis, the gold standard of blood culture can take up to 6 weeks, is not sensitive (20–53%) and, most importantly, not available in most settings in Africa.^{2,5,7} Thus, serology remains the method of choice and can confirm the diagnosis if specimens are collected early and paired sera obtainable. Often in such settings as this, single serum specimen collected several weeks to months after the acute illness is the most readily available specimen. In addition, some serological assays for AFIs often have problems of cross-reactivity with other pathogens⁸ and are difficult to interpret, especially when antibodies persist for a long time, as was seen in this investigation with *Brucella* and *Leptospira*.^{9,10}

Brucellosis was the most likely cause of this outbreak; four of the patients had BMAT results indicative of recent infection. Because of the widespread practice of drinking unpasteurized milk, brucellosis is likely a common, often misdiagnosed cause of AFI in northeastern Kenya. Although brucellosis was first documented in Kenya in the 1950s,¹¹ little is known about its epidemiology in East Africa. Serosurvey data from the 1990s using ELISA with a cut-off value at 1:800, showed that 35% of Nairobi prostitutes and 7% of residents in central Kenya had antibodies to *Brucella*^{12,13}; there is no information on brucellosis among pastoralists in Kenya. Among a pastoralist population in Chad, *Brucella* seroprevalence was 3.8% using tube agglutination and complement fixation tests.¹⁴

In the context of a clinically compatible illness, brucellosis is confirmed by a 4-fold or greater rise in *Brucella* agglutination titer between acute- and convalescent-phase serum specimens obtained at least 2 weeks apart, isolation of *Brucellae* in culture, or demonstration of the organism by specific immunohistochemical staining.¹⁵ Where only a single specimen is available, diagnosis of acute infection is based on a 4-fold reduction in titer between unreduced and reduced BMAT. Using the tube agglutination test, unreduced *Brucella* antibodies may persist at titers \geq 1:320 for over 2 years after illness onset.⁹ The tube agglutination test, the Rose-Bengal test, and other serologic tests all suffer from low specificity, are labor- and time-intensive, and often cannot distinguish acute from remote infection.⁷ The ELISA has been reported to be superior to both culture and tube agglutination in ease of performance, but with varying sensitivities and limited specificity data for diagnosis of brucellosis, the methodology and

interpretation has not been standardized and is prone to false positive results.^{6,16–18} Although the ELISA used here has been shown to have high sensitivity (97%) and specificity (98%)³ when it was compared with blood culture and tube agglutination in a large surveillance population in Egypt, it did not mitigate the problem of interpretation associated with ELISA assays. In this investigation, there was no significant difference in the IgM and IgG ELISA titers, except for specimens from three patients, two of whom had IgM titers several-folds higher than IgG suggestive of acute infection; the other positive IgG and IgM results that were the same or within one-fold titer of each other are difficult to interpret, especially in light of the fact that IgM declines faster than IgG following acute infection.¹⁹ Although polymerase chain reaction is sensitive for *Brucella*,^{20–24} it is expensive, susceptible to false positives, and still not validated for direct use on blood specimens.²⁰

Human leptospirosis has been documented in several parts of Kenya through clinical studies in the 1970s including isolation of leptospires from patients, which is definitive, and surveys in the 1960s.^{25–27} However, these did not include the arid northeastern region where this outbreak occurred and no recent data exists for this region. It is possible that three patients suffered from leptospirosis instead of brucellosis, as leptospirosis may not be endemic in arid Northeast Kenya so that IgM antibodies might reflect acute infection. However, alternate explanations for the leptospirosis IgM are possible. One case had antibodies to both *Brucella* and *Leptospira*, which could have been caused by persisting antibodies from previous infection. Antibodies to *Leptospira*, as well as *Brucella*, are known to persist for more than a year,^{9,10,28} including IgM in the case of *Leptospira*, which occurs in acute infection and was shown to remain detectable up to 3 years after onset (Ari MD and others, unpublished data).

Acute febrile illness is a nonspecific syndrome, caused by an array of pathogens in Africa. To test for the many pathogens requires extensive laboratory tests. Multiplex assays (e.g., polymerase chain reaction and serology) have the advantage of testing multiple pathogens simultaneously, which are usually not available in such developing country settings because of the specialized machines and reagents needed. Even if multiplex assays were readily available for serologic testing of multiple pathogens, this outbreak highlights the difficulty in interpreting such tests. Assays that fail to differentiate one etiology from the other (e.g., brucellosis from leptospirosis) or ongoing active infection from previous exposures ultimately might cause misdiagnosis of outbreaks. Improvements in both detection and differentiation are needed if we are to have a better armamentarium of laboratory tests to use in such outbreaks.

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