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A Foodborne Outbreak of Brucellosis at a Police Station Cafeteria, Lima, Peru

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Abstract. *Brucella melitensis* is highly infectious for humans and can be transmitted to humans in a number of epidemiological contexts. Within the context of an ongoing brucellosis surveillance project, an outbreak at a Peruvian police officer cafeteria was discovered, which led to active surveillance (serology, blood culture) for additional cases among 49 police officers who had also eaten there. The cohort was followed up to 18 months regardless of treatment or symptoms. Active surveillance estimated the attack rate at 26.5% (13 of 49). Blood cultures from four cases were positive; these isolates were indistinguishable using multiple locus variable number tandem repeat analysis. This investigation indicates the importance of case tracking and active surveillance for brucellosis in the context of potential common source exposure. These results provide rationale for public health investigations of brucellosis index cases including the bioterrorism-related dissemination of *Brucella*.

INTRODUCTION

Brucellosis is a zoonotic infection of livestock, primarily cattle, goats, and sheep, and is transmitted to humans by consumption of their raw milk, unpasteurized dairy products, direct contact with infected animals, or secretions from reproductive organs.[1,2] *Brucella melitensis* infections in humans most commonly occur in the context of eating unpasteurized goat (or sheep) milk or cheese products; *B. melitensis* is almost entirely the cause of human brucellosis identified in Peru. In clinical microbiology laboratories, *Brucella* is well known to be highly infectious (with an infective dose of as few as 10 organisms by inhalation) and, being an occupational health risk, prevention of occupationally acquired brucellosis requires high levels of biological safety to protect laboratory and other workers.[3–9] Brucellosis most often presents as a subacute, recurring fever (undulant fever) or may have diverse clinical expressions including meningitis, endocarditis, hepatitis, splenic abscess, arthritis, epididymo-orchitis, spondylitis, focal brain lesions, and other chronic complications including difficult-to-diagnose chronic fatigue-like syndromes.[1] Because *Brucella* spp. are so infectious by the inhalational route they are considered potential agents of bioterrorism and are classified as Category B select agents in the United States.

Brucellosis became established as a focus of endemic country research in Lima, Peru from the 1980s onward. In this context, a prospective, referral-based surveillance and diagnostics development project was carried out in Lima, Peru from 2007 to 2009 that identified new *B. melitensis* antigens useful for serological diagnosis of brucellosis[10–12] and allowed for a larger study catchment area than previously feasible. Recent years have seen a significant reduction in brucellosis cases in Peru as reported by the Ministry of Health Office of General Epidemiology (unpublished data). This decline is thought to be a result of goat vaccination campaigns and other animal public health programs. Human brucellosis still continues to occur in this country and more than 800 cases were reported as recently as 2008 with the majority reported from the Lima metropolitan area[13] where almost half of the country's population lives. Many small, family-based goat farms are located around Lima. Goat white cheese, "*queso fresco*," prepared from unpasteurized milk and traded from distantly located farms is commonly sold in markets in Lima, and is an important source of *Brucella* infection. In Peru, brucellosis affects all socio-economic classes because the consumption of goat's milk products is common. Commercial white cheese production is supposed to be regulated to be made from pasteurized milk to prevent transmission of *Brucella* spp. or other bacterial infection to humans; this is true in large formal markets but not in informal sales venues such as small farms that sell directly to street and informal market vendors. White cheese is often eaten with bread or mixed with other ingredients in the sauces of typical local dishes such as "*Papa a la huancaína*" without cooking.

In December 2007, an outbreak of brucellosis affected officers at a police station in Lima as became manifest by several index cases reported by a local Lima hospital. Three months after the first case, all officers at this police station were serologically screened for brucellosis and additional cases were identified. The outbreak was suspected to be caused by consumption of food made of unpasteurized goat white cheese served in meals at the police station's cafeteria. The results of this investigation show the importance of contact tracing for cases of brucellosis and reinforce the need to regulate unpasteurized goat milk products.

METHODS

Outbreak context and subjects. At the end of December 2007, a group of police officers working at a station in Lima, Peru reported symptoms of joint pain, muscle pain, and excessive sweating. By February 2008, 13 of 60 officers working in

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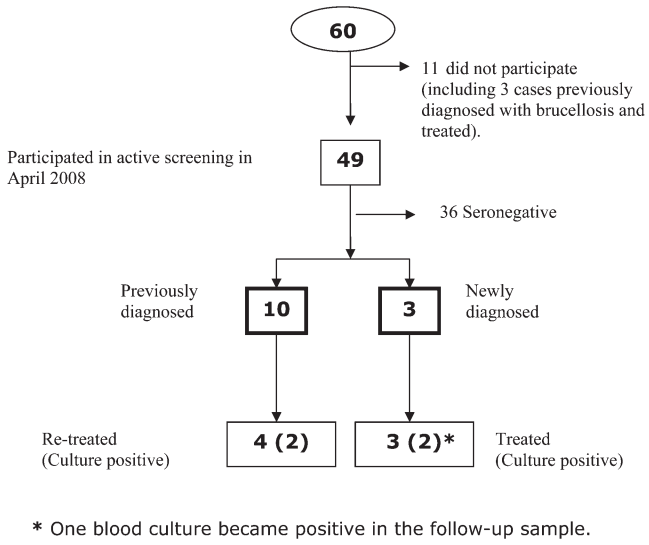

FIGURE 1. Distribution of cases with brucellosis in police station officers from December 2007 to April 2008.

the same office were diagnosed with acute brucellosis based on clinical symptoms, signs, and positive screening serology (positive Rose Bengal [RB] agglutination test). No further laboratory testing at a local health post or police hospital (Hospital de Policia, Lima) was done. Only those presenting to a local health clinic were initially evaluated. In March 2008, four officers originally diagnosed with brucellosis from this station presented to Hospital Nacional Arzobispo Loayza in Lima to seek testing for brucellosis because of persisting symptoms after prior treatment (Figure 1). All were serologically positive, and two were clinically suspected to have active disease based on symptoms, which prompted active screening of all the officers at the police station in early April 2008.

Standard practice by the officers included two work shifts daily and three meals provided in a cafeteria. Sixty officers, including the 13 previously diagnosed with brucellosis, were invited to be screened for brucellosis. The screening was conducted at the police station.

Ethical approval. This study was approved by the Institutional Review Boards of Universidad Peruana Cayetano Heredia, Hospital Nacional Cayetano Heredia, University of California San Diego, Asociación Benéfica PRISMA, Hospital Nacional Arzobispo Loayza, and United States Naval Medical Research Unit No. 6 (NAMRU-6). A project nurse discussed involvement in the project in detail with each potential subject, and all participants provided written informed consent.

Screening tests for brucellosis. The standard RB test[14–17] (Instituto Nacional de Salud, Lima, Peru) and a lateral flow assay (LFA) for detection of specific immunoglobulin M (IgM) and IgG antibodies[18] were used for screening; a positive result on either test was sufficient to be considered positive. Comprehensive clinical and demographic data including personal history of brucellosis and white cheese consumption were recorded. Details of food consumption at the cafeteria were not queried because of the introduction of recall bias.

Confirmatory testing for brucellosis. Screening serological tests were confirmed using the *Brucella* tube agglutination test (TAT) (definitive positive, ≥ 1/160) and the 2-mercaptoethanol (2-ME) *Brucella* agglutination test (definitive positive, ≥ 1/20).[19]

Blood cultures to attempt *Brucella* isolation were done on patients with positive screening serologies, using both the lysis centrifugation method[20] and the BD BACTEC system (Becton Dickinson and Company, Franklin Lakes, NJ).[21] A positive culture was considered definitive proof of brucellosis.

Follow-up. Confirmatory serological tests and blood culture were repeated at 3, 6, and 12 months after completion of treatment. Patients diagnosed with brucellosis in this study were referred to their own physician for treatment and clinical care.

***Brucella* genotyping.** Culture isolates were genotyped independently by laboratories at the Universidad Peruana Cayetano Heredia and NAMRU-6 (Lima, Peru) using multiple locus variable number tandem repeat analysis (MLVA-16 locus).[22,23] Isolates obtained during follow-up were tested using the previously described five polymorphic loci in Peruvian *B. melitensis* biovar 1 isolates (Bruce 07, 09, 16, 18, and 42).[23] A 100 bp and a 20 bp molecular marker ladder (Invitrogen, Carlsbad, CA) were used to estimate MLVA band sizes; the *B. melitensis* strain 16 M was used as the control.

Statistics. Serology and culture results were compared between the symptomatic and asymptomatic groups. Frequencies of background factors (consumption of white cheese outside of the working place and time shift of the work) were compared between seropositive and negative groups. The χ^2 tests were performed using STATA 8 (StataCorp, College Station, TX).

RESULTS

Study participants. Forty-nine (45 men, 4 women, median age: 44 [23–56]) of 60 officers (82%) working at the police station were screened in April 2008, 3 months after the first indication of the outbreak (Figure 1). Eleven officers, including three previously diagnosed cases did not participate in the study, because they either had left the service or declined for personal reasons, therefore they were excluded from the analysis. Ten (20.4%) out of 49 officers who participated in our study reported having been diagnosed with brucellosis between December 2007 and February 2008 (Table 1) before the active surveillance began.

Diagnosis of brucellosis. Three new patients in addition to the already identified 10 patients, out of 49 participants, were

TABLE 1
Characteristics of participants screened in Lima, Peru police station—April 2008

	Screened $N = 49$
Median age (years)	44 [23–56]
Sex (Men:Women)	45:4
Brucellosis previously diagnosed in:	
Nov 2007 or before (before the outbreak)	0 (0.0%)
Dec 2007–Jan 2008 (outbreak)	10 (20.4%)
No history	39 (79.6%)
Presence of symptoms at screening	
Asymptomatic	26 (53.1%)
Symptomatic	23 (46.9%)
Fever	5 (10.2%)
Sweating	9 (18.4%)
Myalgia	11 (22.4%)
Arthralgia	18 (36.7%)

TABLE 2
Laboratory results of participants screened in police station—April 2008*

Laboratory tests	Participants			*P* value
	Total	Symptomatic†	Asymptomatic	
Screened	*N* = 49 (%)	*N* = 23 (%)	*N* = 26 (%)	
Rose Bengal test positive	12/49 (24.5)	10/23 (43.5)	2/26 (7.7)	**0.004**
Lateral flow assay				
IgM positive	9/49 (18.4)	8/23 (34.8)	1/26 (3.8)	**0.005**
IgG positive	11/49 (22.4)	8/23 (34.8)	3/26 (11.5)	0.051
# Screening positive‡	13/49 (26.5)	10/23 (43.5)	3/26 (11.5)	**0.012**
Confirmatory tests§	*N* = 13 (%)	*N* = 10 (%)	*N* = 3 (%)	
TAT ≥ 1: 160	9/13 (69.2)	8/10 (80.0)	1/3 (33.3)	0.125
2-ME positive	10/13 (76.9)	8/10 (80.0)	2/3 (66.6)	0.631
Blood culture positive	4/13 (30.8)¶	2/10 (20.0)	2/3 (66.6)	0.125
# Brucellosis diagnosed‖	13/13 (100.0)	10/10 (100.0)	3/3 (100.0)	–

*TAT = tube agglutination test; 2-ME = 2-mercaptoethanol agglutination test.
† At time of screening, there were 23 symptomatic subjects, manifesting as fever (5), sweats (9), myalgia (11), and arthralgia (18).
‡ Any of the screening tests positive.
§ Applied only for participants with positive screening results.
¶ One culture sample became positive after 3 months from the treatment. It was from an asymptomatic subject (follow-up sample).
‖ Any of TAT, 2-ME, or culture positive, including active and inactive.

RB screening test positive (Table 2). Three of 13 officers with a positive screening test had a positive blood culture and confirmatory serology, which was the basis of identifying three new patients; 9 of 13 had positive quantitative serology (TAT or 2-ME) and negative culture. One blood culture positive case had positive lateral flow testing for IgG but negative TAT and 2-ME tests. Thus, all 13 individuals with positive screening tests were confirmed to have active or past brucellosis infection either by blood culture or quantitative serology tests. Comparison of all symptomatic and asymptomatic participants showed that positivity of each screening test was significantly associated with being symptomatic (Table 2). There was no difference, however, in positivity of blood culture or confirmatory serological tests between asymptomatic and symptomatic cases when compared only in individuals screened as positive (Table 2).

These data indicate that 3 of 13 cases had never been previously diagnosed with brucellosis but were detected by active case finding. Two of these cases had clinical symptoms typical of brucellosis; one remained asymptomatic (Figure 2). Nine of 10 officers identified by a positive RB test were confirmed by quantitative serology except one who only had a positive LFA and a positive blood culture.

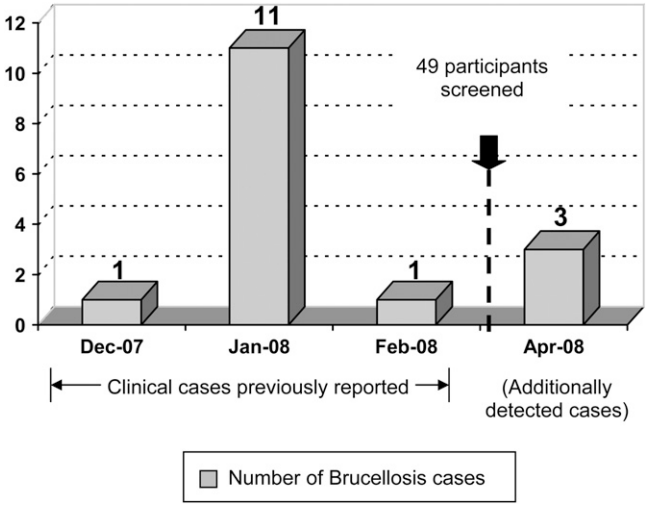

FIGURE 2. Incidence of brucellosis in 60 police station officers in Lima, Peru, December 2007 to April 2008.

Identification of presumed point source of infection. Because all officers consumed their meals at the cafeteria with a limited menu, we presumed that most of the participants ate the same meals if their time shifts were the same. Regarding the consumption of dairy products outside the police station, 11 of 13 seropositive participants (84.6%) reported eating white cheese at the supermarket and 12 (92.3%) consumed "*Papa a la huancaina*" at restaurants and/or at home (Table 3). None reported eating unpasteurized goat white cheese. Regardless, the rates of positive responses for these questions were similar in both seropositive and negative participants (Table 3).

Notably, of the 13 seropositive cases, 11 were officers of the first shift (the odd days of the month) and two were officers of the second shift (the even days of the month). The proportion of the seropositive cases tended to be larger in the officers of first shift (11 of 31; 35.5%) than that in the second shift (2 of 18; 11.1%), although it did not reach statistical significance ($P = 0.06$) (Table 3). All participants received meals at the police station cafeteria that were prepared by a contractor. The participants reported that no family member was known to be affected by brucellosis during or after the outbreak and throughout the duration of follow-up.

Follow-up. Clinical features of the 13 patients of brucellosis were typical of the disease (Table 4). Three newly

TABLE 3
Consumption (intake) of dairy food outside police station and working shift reported during the outbreak

	Brucella serology		
	Positive *N* = 13	Negative *N* = 36	
	n (%)	n (%)	*P* value*
Consumption (intake) of dairy outside of the working station			
Any white cheese (*queso fresco*)	11 (84.6)	30 (83.3)	0.915
Non-pasteurized white cheese	2 (15.4)	4 (11.1)	0.678
Any goat cheese	0 (0.0)	1 (2.8)	0.443
Papa a la Huancaína	12 (92.3)	35 (97.2)	0.443
Working shift			
Group 1 (*N* = 31)	11/31(35.5)	20/31 (64.5)	
Group 2 (*N* = 18)	2/18(11.1)	16/18 (88.9)	0.062

* *P* values are from χ^2 test used for comparison of two groups.

TABLE 4
Clinical features of 13 patients with brucellosis after police station common source outbreak*

Case (age/sex)	During the outbreak		3 months after the outbreak			Comments
	Brucellosis diagnosis	Treatment regimen	Symptoms	Blood culture	Treatment regimen	
1 (42/F)	Yes	Doxy + Rif	Arthralgia	+	Rif + Gent + Azi	6 weeks pregnancy. Seronegative 12 months after treatment.
2 (37/M)	Yes	Did not recall	None	+	1° Not treated 2° Doxy + Rif	14-day treatment during outbreak. On screening, culture positive, asymptomatic, declined treatment. After 3 months (July 2008), developed fever, arthralgia, and received treatment. Seronegative at 12 months after treatment.
3 (50/M)	No	No	Sweats, headache, chills, arthralgia	+	Doxy + Rif	Seronegative 6 months after treatment.
4 (45/M)	No	No	None	+	1° Doxy + Rif 2° Doxy + Rif	Relapse with positive culture without symptoms 3 months after 1st treatment. Seronegative at 18 months after 2nd treatment.
5 (47/M)	Yes	Doxy +Rif	Fever, myalgia, arthralgia	–	1° Doxy 2° Doxy + Amik	Retreated for relapsed symptoms 5 months after 1st treatment after outbreak. Seronegative at 12 months after 2nd treatment.
6 (48/M)	Yes	Doxy + Rif	None	–	Not treated	Seronegative 7 months after the outbreak.
7 (48/M)	Yes	Doxy +Rif	Headache, lumbar pain	–	Not treated	Seronegative 19 months after the outbreak.
8 (56/M)	Yes	Doxy + Rif	Weight loss, headache, arthralgia, lumbar pain	–	Doxy + Rif + Gent	12-month follow-up ended with positive serology but no symptoms.
9 (42/M)	Yes	Doxy + Rif	Weight loss, headache, arthralgia, diarrhea, insomnia	–	Not treated	Seronegative 15 months after the outbreak.
10 (44/M)	No	No	Sweats, headache, myalgias, arthralgia, lumbar pain	–	Doxy + Rif	Seronegative after the treatment until completion of follow-up after 12 months.
11 (46/M)	Yes	Doxy + Rif + Trime-Sulfa	Weight loss, myalgias, arthralgia	–	Not treated	Seronegative 15 months after the outbreak.
12 (49/M)	Yes	Doxy + Rif	Sweats, headache, myalgias, arthralgia, lumbar pain, diarrhea	–	Not treated	Seronegative 9 months after the outbreak.
13 (39/M)	Yes	Doxy + Rif	Sweats, general malaise, myalgias, arthralgia	–	Not treated	Followed at another hospital so no follow-up data available. No symptoms 18 months after the outbreak.

*Ami = Amikacin; Azi = Azithromycin; Doxy = Doxycycline; Gent = Gentamicin; Rif = Rifampin; Trime-Sulfa = Trimethoprim-Sulfamethoxazole.

diagnosed brucellosis cases received a full 45-day course of standard antibiotic treatment (doxycycline plus rifampin). Among the 10 previously diagnosed and treated cases, four were retreated (Table 4). Twelve of 13 cases were followed for 18 months after the outbreak. On follow-up, two cases relapsed after treatment; one was identified by blood culture, the other by an increased titer in the serological tests (TAT and 2-ME). Both cases had symptoms consistent with brucellosis, including headache, myalgia, arthralgia, and insomnia. The other 10 cases appeared clinically cured after completing treatment and had gradual declining titers without reporting any symptoms of brucellosis throughout the follow-up period. The remaining patient (who was not followed) was contacted more recently and denied relapse of the symptoms.

Genotyping of *Brucella* isolates. All four *Brucella* isolates had an identical MLVA type (Figure 3), which is the most frequently reported genotype among human *B. melitensis* biovar 1 isolates isolated in the period 2003–2007 from patients from Lima.[23]

DISCUSSION

To our knowledge, there are no reports of a work-associated outbreak of brucellosis affecting people not directly dealing with animal products or bacteria. Previous reports focused on family,[24–27] laboratory, or animal-associated risk factors for infection.[3–9] Food-borne outbreaks have been reported in families or neighborhoods as a result of the ingestion of goat white cheese distributed to households.[6,24–28] It is known that patients with *Brucella* infection may well be asymptomatic despite being proven by culture to be bacteremic.[29,30] Nonetheless, our present report reinforces the need for active surveillance and contact tracing after apparent point source outbreaks given the subclinical or early cases that are not identified in a timely manner because of non-specific symptoms. This outbreak also reinforces the need to consider different cultural issues regarding potential food point exposures in brucellosis transmission. Our characterization here of occupation-related brucellosis reflects the practice among police officers in Peru to share meals, whereas brucellosis acquisition in the occupational context typically refers to agricultural or veterinary exposure.

It is most likely that food ingested at the police station cafeteria was responsible for transmission of *B. melitensis*. This assumption is not only based on the monomorphic MLVA findings in the few isolates obtained from cases, but also based on finding additional cases associated primarily with one working shift. None of the household members of the cases developed brucellosis during the 18 months of our follow-up. In previous studies, however, a high rate of family cases has been found in household members of brucellosis patients,[24,25,27,28] suggesting that the infection was not acquired at home in this outbreak.

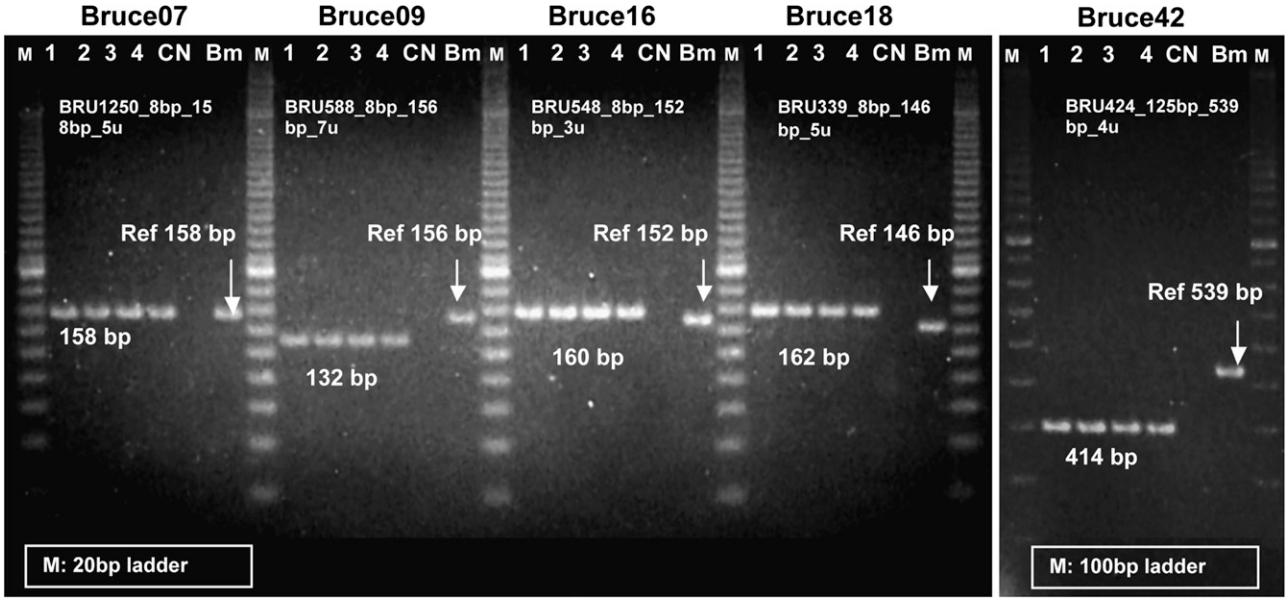

FIGURE 3. Multiple locus VNTR genotyping of *Brucella melitensis* strains from four isolates obtained from April 2008 to August 2008.* * M:Marker; 1, Case 1; 2, Case 2; 3, Case 3; 4, Case 4 (Relapsed); CN, Negative control; Bm, *Brucella melitensis* 16M strain.

Some local Peruvian cuisine, "*Papa a la huancaina*," "*Ocopa*," and "*Chupe*, prepared with unpasteurized goat white cheese might have been the source of the acquired infection. It was known that these dishes were served to all before the outbreak and have characteristics of being eaten without the consumer knowing whether unsafe ingredients were present in the food. However, our interviews were performed 3 months after the outbreak, making dietary history imperfectly reliable in this study.

Of 60 officers at the police station, 13 (21.7%) sought medical attention before our study and were diagnosed with brucellosis. After 3 months, we identified three additional cases. This outbreak resembles a previous study of laboratory-acquired *Brucella abortus* infection followed by sequential active serological tests, which showed that most of the infected individuals were asymptomatic when they seroconverted with time to seroconversion varying from 6 weeks to 5 months[8]; a similar range of the incubation period was reported in the case of *B. melitensis* infection.[9]

Although regular serological surveillance is done on laboratory workers handling *Brucella*, active testing has not been reported in the setting of potential food exposures. Several studies of family-based outbreaks have reported asymptomatic seropositive or bacteremic cases found during active surveillance.[6,28]

Because the incubation period of human brucellosis is highly variable, our study reinforces the clinical need to serially test people at risk for *Brucella* infection following potential or known exposures, even if asymptomatic. For laboratory exposure, it is recommended to test weekly or semiweekly for the first 3 months using standard serologies, and once a month thereafter for 3 to 9 months after exposure. Prophylaxis after assessment of exposure risk is carried out typically using doxycycline 200 mg/day plus rifampin 600 mg/day for at least 3 weeks.[4] Insufficient evidence exists to support administering antibiotic prophylaxis after possible ingestion of *Brucella*-containing foods, and the culture or molecular analysis of food for *Brucella* is difficult and not feasible. The data provided here would support a recommendation to follow patients with serological tests and blood cultures to detect subclinical infections or later manifestations of brucellosis.

In our patient cohort, 2 of 13 (15%) relapsed after 3 and 6 months despite completing standard antimicrobial therapy. These relapsed patients were treated with rifampin and doxycycline for an additional 6 weeks according to the recommendations by the World Health Organization (WHO).[9] Relapse rates have been reported to range from 10% to 20%[1,4] and were similar to ours. Follow-up after treatment is recommended as previously described.[4]

Part of this study was retrospective, with some important limitations. First, the standard serological tests that we used to identify brucellosis cases, although reported to be > 90% sensitive and specific,[2,14,31,32] nonetheless are imperfect; the gold standard diagnosis is isolation of the organism in culture. The reason that the RB and lateral flow assay were chosen as screening tests in this study was to take advantage of the relatively high sensitivity of these tests to enhance the chances of capturing cases. The RB test used to screen enrolled subjects could have over-estimated cases because previous infection unrelated to this outbreak may have led to seropositivity. There is a theoretical risk of the *Brucella* agglutination tests cross-reacting with antibodies to *Yersinia enterocolitica*, *Francisella tularensis*, and *Escherichia coli* O157, which in the present context is not clinically or epidemiologically consistent. The lateral flow assay used as a screening test in this study—which detects IgM and IgG antibodies to *Brucella* LPS—would potentially have the same limitation; the prolonged incubation time of brucellosis can lead to both diagnostic titers of IgM and IgG antibodies at the time of presentation, and, in the case of this epidemiological investigation, at the time of screening. However, previous studies have shown that even in endemic regions the LFA IgM/IgM test has a sensitivity and specificity of ~> 96%, even when used in endemic areas.[18,33,34] The performance of screening

agglutination tests (either the RB or slide agglutination test) occasionally is inferior to the tube agglutination test, which has different incubation times and conditions.[35] Confirmatory serologies performed in this study included the tube agglutination test (with a 1 of 160 titer cutoff) and the 2-ME test (that differentiates IgM from IgG antibodies, indicating acute infection) to enhance the specificity of testing; previous studies from Peru and elsewhere have shown these criteria to be strongly suggestive of a new episode of brucellosis.[19,27] Tests to detect non-agglutinating anti-*Brucella* antibodies using the Coombs or BrucellaCapt test were not used in this study, which may have led to an underestimation of cases. Overall, the combination of serological tests done in this study served to enhance both the sensitivity and specificity of active case finding among the potentially affected population. Another limitation of this partially retrospective study is that written laboratory test results of the officers affected at the beginning of the outbreak (December 2007) were not available, therefore we had only verbal information from them regarding their prior diagnosis and treatment of brucellosis. Detailed information was not sought regarding the frequency of ingestion of high risk foods at the cafeteria because of the need to avoid recall bias. Nor could we obtain food distribution sources from the cafeteria contractor. Culture samples of the meals served at the cafeteria were not obtained to look for *Brucella*, which numerous studies have shown to be highly challenging and largely unrewarding. Another limitation of this study is that differentiating infecting *B. melitensis* strains remains challenging. The standard MLVA panel for *Brucella* uses 16 loci and thus the monomorphic results may not differentiate subtle differences; in this study only five loci used have proven useful for differentiating among strains.[23] Whole genome sequencing would be necessary to investigate more detailed differences between infecting strains. Finally, there is a remote possibility that the identified relapses may have been new infections.

CONCLUSIONS

The clinical pathogenesis of brucellosis depends on multiple, mostly unknown interactions between pathogen and host that combine to prolong the time from exposure to clinical manifestations. Active case finding and follow-up after potential point-source foodborne exposures, as indicated by sentinel cases should be carried out despite the absence of symptoms. An unanswered question is whether prophylactic treatment is beneficial for those who shared the dishes during the outbreaks. Finally, food safety monitoring and regulated pasteurization of dairy products are necessary for the control of brucellosis. Improved monitoring will require reliable culture or bacterial antigen detection methods for food tracking and the active screening of milk products and carrier animals in areas where brucellosis persists.

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