Am. J. Trop. Med. Hyg., 87(3), 2012, pp. 524–528
doi:10.4269/ajtmh.2012.11-0341

Case Report: First Report of Orchitis in Man Caused by *Brucella abortus* Biovar 1 in Ecuador

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Abstract. We present a 44-year-old man from a rural community in northern Ecuador who worked on a cattle farm where he was involved with primary veterinary care, including assistance during births (or calving) and placenta retention and artificial insemination, with minimal precautions. In September of 2009, quite abruptly, he developed asthenia and hypersomnia without any apparent cause or symptoms like fever, chills, or night sweats. On November 14, 2009, he suffered from pain and edema in the right testicle that coincided with pain in the abdomen. Clinical, serological, and bacteriological investigations confirmed the first case of unilateral orchitis in man in Ecuador caused by *Brucella abortus* biovar 1. Because brucellosis is a neglected disease, special attention should be given to it in the training of medical and veterinary students.

INTRODUCTION

Brucellosis is a bacterial infection with severe repercussions on human and animal health, causing heavy losses worldwide.[1] In man, it can start as an acute disease with recurrent fever, sweating, general discomfort, headache, muscle pain, and arthritis. The infection is acquired through direct or indirect contact with infectious material from animal origin (i.e., aborted fetuses, placentas, and vaginal secretions) as well as accidental inoculation of vaccine, inhalation of aerosols, or consumption of non-pasteurized dairy products (mostly affecting slaughterhouse or laboratory staff).[2]

The frequency of human infections is directly related to the prevalence in animal reservoirs.[3] In countries where adequate control or eradication programs are implemented, this zoonosis is a professional hazard, whereas in developing countries, the entire population is at risk, including tourists or other visitors of contaminated ranches.[4]

Complications such as spondilytis, arthritis, hepatosplenomegaly, and endocarditis are often observed in humans with genitourinary inflammations (epididymitis, orchitis, and prostatitis) reported in 2–20% of positive cases.[5] *Brucella melitensis* is reported to be the most pathogenic species in man and responsible for most of the clinical cases in man.

According to official reports, the annual incidence of human brucellosis in Ecuador remains rather low (i.e., between 1990 and 2007, it affected no more than 0.21 per 100,000 persons),[6] whereas in the same time span, in Carchi (the province where the present case has been described), four positive cases were detected (0.62 per 100,000 persons).[6] In the absence of an official reference laboratory in Ecuador, routine isolation and biotyping of *Brucella* spp. are not done; for this reason, the existence of possible reservoirs remained completely unknown.

In contrast, preliminary studies by the International Center for Zoonosis (CIZ) suggest that the seroprevalence of brucellosis in man can be around 2% in the northwestern provinces of Ecuador. The main risk factors are the consumption of boiled milk (odds ratio [OR] = 4.69, confidence interval [CI] = 1.45–15.18) or raw milk (OR = 5.13, CI = 1.52–17.29) and contact with fetal and placental tissue (OR = 2.10, CI = 1.02–4.33) from cattle (Ron-Román J and others, unpublished data).

CASE REPORT

A male patient (44 years old, married, and born and living in Cuaspud, a rural community in Huaca canton, Carchi province, in the north of Ecuador) worked as a cattle farm laborer for the last 3 years. As such, he was actively involved with primary veterinary healthcare, including assistance during births and placenta retention and artificial insemination in the cantons Huaca, Montufar, Bolívar, and Tulcán in Carchi province with minimal hygienic precautions.

In September of 2009, quite abruptly, he developed asthenia and hypersomnia without any apparent cause or symptoms like fever, chills, or night sweats. On November 14, 2009, he suffered from pain and oedema in the right testicle and abdominal pain. He consulted a general practitioner in the city of Tulcán who empirically prescribed 75 mg sodium diclofenac (during 7 days), 10 mg sodium naproxen (during 7 days), and 200 mg cefpodoxime (during 7 days). This treatment, however, brought no relief, and as suggested by a veterinarian, a brucellosis agglutination test (Febrile Antigen Agglutination Test; Becton Dickinson, Sparks, MD) was performed in a private laboratory in Tulcán. This test showed a positive titer of 1/320 (cutoff ≥ 1/160) for *Brucella* spp. On November 22, he started to suffer from lumbalgia, testicular edema, and generalized asthenia. During this period, he had unprotected sex with his wife.

On November 23, he came to the CIZ of Central University of Ecuador with the diagnosis of unilateral right orchitis, lumbosacral pains, general asthenia, and positive diagnosis of brucellosis by the Febrile Antigen Agglutination Test (Becton Dickinson).

At CIZ, two serological tests showed these results: fast agglutination plate assay was Rose Bengal (RB) positive (++), and Wright's slow agglutination test with ethylenediaminetetraacetic acid (SAT-EDTA) was positive (1,280 international units of agglutination [IAU], dilution 1/800, cutoff ≥ 1/25). On November 26, a blood culture (4 days incubation; Bactec System) was positive for the presence of *Brucella* spp. in Hospital Vozandes Quito (HVQ).

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TABLE 1
Case report of a patient with brucellosis-related epididymo-orchitis: Hematological and biochemical values

	Results				
	12/3/2009	12/17/2009	1/8/2010	Units	Reference range
Sedimentation rate	24*	5	ND	mm/hour	0–20
Leukocytes	7.93	5.22	5.06	K/μL	4.80–10.80
Neutrophils	52.0	41.2*	40.9*	%	43.0–65.0
Lymphocytes	35.3	50.6*	50.0*	%	20.5–45.5
Monocytes	12.0*	6.3	7.1	%	1.9–9.0
Eosinophils	0.4*	1.1	1.4	%	1.0–5.0
Basophils	0.3	0.8	0.6	%	0.2–1.0
Erythrocytes	5.36	5.52	5.51	M/μL	4.5–6.0
Hemoglobin	16.0	16.5	16.4	g/dL	14.0–18.0
Hematocrit	47.3	47.7	48.6	%	41.0–51.0
Mean corpuscular volume	88.2	86.4	88.2	fL	80.0–90.0
Mean corpuscular HB	29.9	29.9	29.8	pg	27.0–31.0
Mean corpuscular HB concentration	33.8	34.6	33.7	g/dL	32–36
Distribution width RDW SD	42.8	41.6	43.0	fL	37.0–54.0
Distribution width RDW CV	14.1	13.9	14.0	%	11.5–15.5
Platelets	308	282	252	K/μL	100–500
Mean platelet volume	8.7	8.4	8.9	fL	7.2–9.5
C-reactive protein	56.9*	0.66	0.36	mg/L	0.00–5.00
Creatinine	0.86	0.98	ND	mg/dL	0.70–1.20

* Values outside reference range.
ND = not done; HB = hemoglobin; RDW = red cell distribution width; SD = standard deviation; CV = coefficient variation.

On December 3 in the outpatient clinic of the same hospital, the medical staff opted for the treatment regimen recommended by the World Health Organization[7] of doxycycline (100 mg, BID) for 6 weeks and gentamicin (320 mg intravenously daily) for 10 days. Vital signs seemed to be normal: blood pressure (BP) = 110/60 mmHg, pulse (P) = 71/minute, oral temperature (OT) = 37°C, and weight = 57.5 kg; on rectal examination, no evidence of prostatitis was observed. High values for C-reactive protein (56.9 mg/L; normal values are between 0.00 and 5.00 mg/L) indicated an inflammatory process; hematological, biochemical, and serological results are presented in Table 1.

In the laboratory for microbiology of CIZ, culture of the patient's semen in Farrell's medium (Columbia Blood Agar Base CM0331 [Oxoid] + horse serum [reference 16050–130; Gibco] + modified *Brucella* Selective Supplement SR0209E [Oxoid]) isolated *Brucella* sp. Subsequently, both strains from blood (Ec-CIZ-Hum-6) and semen (Ec-CIZ-Hum-7) were typified as *B. abortus* biovar 1 (field strain) by biochemical assays (Table 2) and Abortus, Melitensis, Ovis and Suis (AMOS)-polymerase chain reaction (Figure 1).[8–10]

Two blood samples (taken on 12/3/2009 and 3/26/2010) of the patient's wife yielded negative results in the RB and SAT-EDTA assays; hence, no sexual transmission occurred.

On December 17 at a clinical follow-up in the hospital, the patient's status was improved, with receding orchitis, low back pain, and asthenia. Vital signs were stable (BP = 100/70 mmHg, P = 78/minute, OT = 37°C, and weight = 61.5 kg). Immunological assays showed the presence of *Brucella* sp. antibodies (RB +++ and SAT-EDTA = 1,280 IAU); *Brucella* sp. remained persistent in the blood circulation as shown by blood culture. Additional results are presented in Table 1. On January 8, 2010, a decrease in circulating immunoglobulin M (IgM) was shown by SAT-EDTA (400 IAU), but the RB assay remained highly positive (+++). Additional values are presented in Table 1. The third visit showed a complete recovery of the patient: BP = 120/90 mmHg, P = 67/minute, OT = 36.5°C, and weight = 60 kg. The patient was declared to be cured, and a monthly serological follow-up to detect relapses was recommended.

As presented in Table 3, the results of the serological assays showed a fast decline of IgM levels (as detected by SAT-EDTA), whereas the levels of IgG (by RB) remained high.

In August of 2010, the patient reported a mild pain in the lumbosacral joint that deteriorated in time. Based on a clinical examination and laboratory results (Table 3) showing a steep rise in IgM and IgG (i.e., by SAT-EDTA on 7/14/2010 and much later on 12/1/2010 by RB), the infectious diseases

TABLE 2
Case report of a patient with brucellosis-related epididymo-orchitis: Characteristics of bacterial isolations

					Growth on colorants				Agglutination with serum	
Bacteriological sample	Sample	Urease activity	CO_2 requirement	H_2S production	Thionin (20 μg)	Thionin (10 μg)	Basic fuschin (20 μg)	Safrani (100 μg)	Anti-A	Anti-M
Ec-CIZ-Hum-6	Blood	+ (20 hour)	− (48 hour)	+ (24 hour)	−	−	+ (6 day)	+ (48 hour)	+	−
Ec-CIZ-Hum-7	Semen	+ (20 hour)	− (48 hour)	+ (24 hour)	−	−	+ (6 day)	+ (48 hour)	+	−
B2*		+	+	+	−	−	−	−	+	−
B9†		+	−	+	+	+	+	+	−	+
B1		+	+‡	+	−	−	+	+	+	−

* Control *B. abortus* biovar 2.
† Control *B. abortus* biovar 9.
‡ Positive for the most of the strains.
EC-CIZ-Hum-6 and -7 are the *Brucella* isolates from the patient.

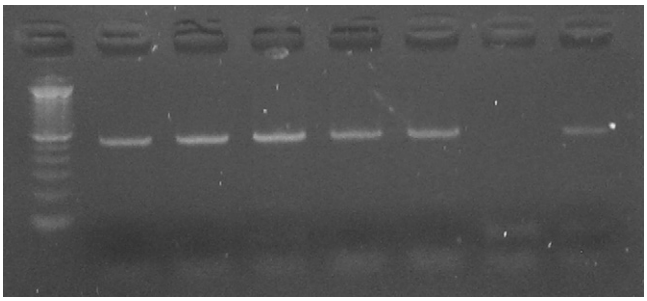

FIGURE 1. Case report of a patient with brucellosis-related epididymo-orchitis: AMOS-polymerase chain reaction results. CN = negative control; CP = positive control; H-19 = human sample 19 (Ec-CIZ-Hum-7); H-21 = human sample 21 (Ec-CIZ-Hum-6); MP = molecular weight marker. *Samples from additional studies at CIZ.

specialist of HVQ decided to start the treatment again to treat a possible relapse.

On November 7, 2010, the following treatment was used: doxycycline (100 mg every 12 hours) for 6 weeks plus gentamicin (320 mg daily intravenously) for 2 weeks. At that time, blood and semen cultures were negative for *Brucella* sp.

DISCUSSION

The official reports concerning the surveillance, control, and eradication of brucellosis in Ecuador state the limited importance of this zoonosis,[6] and as such, these reports are accepted in the international literature[11]; however, they seem to be far from reality. Studies performed by the CIZ show more realistic numbers of cases that are not only in persons living or working in high-risk conditions (Ron-Román J and others, unpublished data).

Epididymo-orchitis is frequently observed in regions where *B. melitensis* is endemic,[12] and it has been described in 2–20% of the infected patients[12–14] (mostly younger patients).[12,14,15]

TABLE 3

Case report of a patient with brucellosis-related epididymo-orchitis: Results of the serological assays

Sample number	Date	RB	SAT-EDTA (IAU)
1	11/23/2009	++	1,280
2	12/17/2009	++	960
3	1/8/2010	++	480
4	2/14/2010	+	160
5	3/26/2010	+	120
6	4/24/2010	+	120
7	5/31/2010	+	100
8	7/14/2010	+	200
9	8/17/2010	+	240
10	9/21/2010	+	400
11	10/18/2010	+	240
12	12/1/2010	++	200
13	1/14/2011	++	200
14	2/11/2011	+	100
15	3/17/2011	+	100
16	4/27/2011	+	100
17	6/6/2011	+	80
18	7/14/2011	+	60
19	8/11/2011	+	60
20	10/21/2011	+	50
21	12/12/2011	+	30

IAU = international units of agglutination; RB = Rose Bengal test; SAT-EDTA = Wright's slow agglutination test with EDTA.

However, the present study is the first report in Ecuador with *B. abortus* as the causative agent.

Usually, brucellosis-related epididymo-orchitis is unilateral,[12,16–19] but bilateral infections have been reported.[20,21] As in the present case, the majority of cases (53–69%) were confirmed by positive blood cultures.[13]

Isolation of *Brucella* by epididymal aspiration was performed in 6.7% of the patients with epididymo-orchitis[22]; in the present study, *B. abortus* was isolated from semen, which warranted the possibility of sexual transmission, but paired serology taken from his wife with an interval of 16 weeks remained negative by RB and SAT-EDTA. The isolation of *B. melitensis* in semen from a patient with epididymo-orchitis has been reported[23]; also, *Brucella* sp. has been isolated from blood culture.[24]

Several works have promoted leucocytosis as an indicator to differentiate epididymo-orchitis caused by *Brucella* spp.[14,15,18,20,25–27]from non-specific orchitis, but it was not observed in this case. In non-specific orchitis, signs of inflammation are very clear (i.e., dermatitis of the scrotal skin). In *Brucella* spp.-induced orchitis, the fever is undulating and lower than in nonspecific orchitis; also, in the present case, the patient never reported fever or chills.[28]

Although the final diagnosis of brucellosis is based on the isolation of the causal agent, usually from blood samples or other bodily fluids, the use of standardized immunodiagnostic assays would be a great contribution to the diagnosis. Cases of brucellosis confirmed by blood culture with negative serological results have been reported, albeit they are rare.[29,30]

When bacterial isolation is negative but serological results are positive or suspected, this zoonosis has to be taken into account, even more so when there is a history of risk, such as contact with natural carriers, dubious hygienic standards, living in an endemic area, or symptoms associated with brucellosis.

In endemic regions, the slightest suspicion should be sufficient to start treatment in anticipation of the confirmation by laboratory tests.[13] Therefore, a detailed anamnesis together with a correct risk assessment is of the utmost importance to underpin the diagnosis and specific treatment of epididymo-orchitis, and it will help to avoid complications such as testicular abscesses or atrophy leading to infertility.[31–33] In patients with epididymo-orchitis, the differential diagnosis should include brucellosis when living in an endemic region such as Ecuador, especially when these patients belong to a high-risk population. In this respect, it is interesting to note that, in 2008, epididymo-orchitis was reported in 983,286 hospitalized patients in Ecuador, but only 369 (0.037%) cases were ascribed to brucellosis (diagnostic codes N.45.0–N.45.9 according to International Statistical Classification of Diseases and Related Health Problems).[34] However, the 369 cases, provisionally attributed to brucellosis, were not confirmed by the isolation of the causal agent because of the lack of a diagnostic strategy and laboratory infrastructure for this zoonosis.

Given the intracellular localization of *Brucella* spp. mostly in the reticulo endotelial cells, the choices of the antibiotic, its dose, and duration of the treatment are crucial. The combined use of antibiotics for the treatment of brucellosis is often recommended, specifically when complications like epididymo-orchitis are involved, and it might help to reduce the chances of relapses.[35] The synergy of several antibiotics (doxycycline, rifampicin, and streptomycin) has shown efficacy in the treatment of this zoonosis.[17]

Relapse of uncomplicated cases has been reported in 5–10% of cases,[36] and it generally occurs weeks or months after the end of the treatment; it should be confirmed by the isolation of *Brucella* from blood or other bodily fluids or tissues.[37] However, in the present case, thanks to the continuous serological follow-up, the event of a possible relapse could be anticipated. As such, a complementary treatment was justified, thus avoiding septicemia and clinical complications.

For RB, the intensity of reaction was expressed as negative (–; absence of reaction) or + to ++++ (degree of agglutination), which *a posteriori* enables comparison with other quantitative assays such as i-enzyme–linked immunosorbent assay.

A possible relapse could not be confirmed by isolation of the causal agent (i.e., a negative blood and semen culture until 10/7/2010), but given the history of orchitis, it was decided to start a second treatment to minimize the risk for a relapse or development of a chronic localized brucellosis. Furthermore, the results of the SAT-EDTA assay (Table 3) and the symptoms reported by the patient underpinned the decision for a second treatment. However, despite the detection of antibodies on 7/14/2010, at that time, the medical specialist did not consider this result to be a sufficient criterion to start a second treatment. It only began when the pain in the lumbosacral region started to rise combined with an increase of antibodies, mainly detected by SAT-EDTA.

The observations made during the follow-up of the present case emphasize the need to accurately identify the causal agent of the disease and not rely solely on symptomatic treatment. As pointed out in previous studies by the CIZ, brucellosis is an important zoonosis in Ecuador; therefore, it is essential that, in primary healthcare, the clinical and serological tools to diagnose this infection should be available. In addition, because it is a neglected disease, special attention should be given to it in the training of medical and veterinary students.

To minimize as much as possible the risk for the human population to contract brucellosis in Ecuador, efforts should be made to control (eradicate) brucellosis in its natural reservoirs, and therefore, a solid control (eradication) program is needed. In anticipation of this program, it is necessary to implement an education program, covering topics related to the transmission, prevention, control, and diagnosis of brucellosis in man and animals, at least for the population at risk and its medical staff. Finally, we want to emphasize that no control or eradication program for the brucellosis in Ecuador will bring satisfactory results at the desired time without the compromise and joint work between the different actors at national level. Medical doctors and veterinarians should focus and shore up the One Health initiative.

Received May 28, 2011. Accepted for publication February 3, 2012.

Acknowledgments: Special thanks to the patient and his wife who participated in the study. We also want to thank to Drs. Carlos Ron Román and Katherine Pérez Rivadeneira for assisting during drafting the paper.

Financial support: This study was supported by the International Center for Zoonoses (CIZ) of Central University of Ecuador and the Belgian Development Agency (BTC-CTB).

Disclaimer: The views expressed in this article are the views of the authors and do not necessarily reflect the official policy or position of the Central University of Ecuador or Hospital Vozandes Quito. Before being included in the study, participants were asked for verbal and written consent. None of the authors has financial or personal conflict of interest related to this study.

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