ORIGINAL ARTICLE

High Incidence of Respiratory Involvement in a Cluster of *Brucella suis*-Infected Workers from a Pork Processing Plant in Argentina

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Impacts

- Human infection by *Brucella suis* is an occupational hazard in pork processing plants and slaughterhouses in countries with high prevalence of swine brucellosis.
- Whereas human cases of *B. suis* infection have been reported in many countries, outbreaks in occupational settings have been rarely informed. Here, we report 17 cases of *B. suis* infection among workers of a pork processing plant occurring along 18 months.
- Although brucellosis usually presents as a febrile illness with hepatic involvement, the unusually high incidence of respiratory involvement found in this series suggests that brucellosis must be considered in the differential diagnosis of respiratory diseases in pork processing plant employees.

Keywords:
Brucella suis; brucellosis; pulmonary; pig; abattoirs; personal protective equipment

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Received for publication April 6, 2016

doi: 10.1111/zph.12339

Summary

Epidemiological and clinical aspects of *Brucella suis* infection in 17 workers from a pork processing plant in Argentina occurring between January 2014 and July 2015 are presented. All patients reported working 9 h daily without adequate personal protection garment. Blood cultures were positive for *Brucella* spp. in 14 of the 17 patients (82.3%). All isolates were identified as *B. suis* biovar 1. Although fever, sweats, asthenia, myalgia and hepatic involvement were the most frequent clinical manifestations, an unusually high incidence of respiratory involvement was found. From 13 patients in which chest radiography was performed, four (30%) had radiological abnormalities, including lobar pneumonia in two cases (one with pleural effusion) and interstitial involvement in other two. The high frequency of respiratory involvement in our series makes necessary to consider brucellosis in the differential diagnosis of respiratory diseases in pork processing plant employees.

Introduction

Brucellosis is a worldwide distributed zoonosis caused by several *Brucella* species, mainly *B. melitensis, B. abortus* and *B. suis*. Humans acquire the infection by direct contact with tissues or secretions from infected animals, consumption of raw meat or unpasteurized milk, or accidental exposure in microbiology laboratories. Whereas most cases are produced by *B. melitensis* and *B. abortus*, in South America and mainly in Argentina *B. suis* infection has been frequently reported (Meirelles-Bartoli et al., 2012; Escobar et al., 2013).

Five *B. suis* biovars have been identified, but only biovars 1, 3 and 4 are important human pathogens. Biovars 1 and 3 are found in pigs, whereas biovar 4 affects reindeer and caribou (Iowa State University, 2009). Serological surveys in Argentina revealed that 15% to 25% of the pigs have anti-*Brucella* antibodies (Samartino, 2002; Castro et al., 2006). In Latin America, biovar 1 has been the main *B. suis* isolate from both animals and humans (Pappas et al., 2006;

Lucero et al., 2008). Human infection by all pathogenic *Brucella* species, including *B. canis*, has been documented in Argentina (Lucero et al., 2008). While cattle vaccination has led to a reduction in the number of human cases due to *B. abortus* in our country, human infections by *B. suis* have increased (Health Ministry of Argentina, 2007), probably due to the lack of control programmes for swine brucellosis.

Individual cases of human *B. suis* infection have been reported worldwide. However, outbreaks in occupational settings were reported less frequently (Hendricks et al., 1962; Buchanan et al., 1974; White et al., 1974; Trout et al., 1995). Notably, similar outbreaks have not been reported in the last 20 years. In this report, we describe epidemiological and clinical aspects of *B. suis* infection in workers from a pork processing plant occurring along 18 months. In this series, in which most patients had detectable bacteraemia, an unusually high incidence of respiratory involvement was found. The high frequency of respiratory involvement in our series makes necessary to consider brucellosis in the differential diagnosis of respiratory diseases in pork processing plant employees.

Materials and Methods

A retrospective study of 17 brucellosis cases involving pork processing plant employees who sought medical care in the 'Francisco J. Muñiz' Hospital between January 2014 and July 2015 was performed. Clinical records provided by treating physicians were reviewed. The study was approved by the Institutional Review Board of the 'Francisco J. Muñiz' Hospital. Written informed consent was obtained from patients during their follow-up visits to this hospital.

Active brucellosis was diagnosed in patients with compatible clinical manifestations and positive blood culture and/or agglutination titres higher than 100. Patients that exhibited clinical recovery after therapy but later presented clinical manifestations associated with positive blood cultures or increasing serological titres were considered relapsing cases.

Automated blood cultures (BacT/ALERT - bioMérieux) were performed in all cases. Isolates were identified as *Brucella* spp. using established biochemical tests. Positive isolates were submitted to the Brucellosis Reference Laboratory (ANLIS-INEI) for biovar typification.

The Rose Bengal test was used as screening serological test for brucellosis for all the plant workers who sought medical care in our hospital. Positive cases were confirmed by the standard tube agglutination test (STA), complement fixation (CF) and competitive ELISA (C-ELISA). Serologically active brucellosis was diagnosed when a positive Rose Bengal test was accompanied by STA $\geq$ 100, CF $\geq$ 10 or C-ELISA $\geq$ 28% (inhibition percentage).

Results

The study included 17 male patients from 28 to 58 years old (mean 35.76, median 34), who had worked in the pork processing plant for 6 months to 18 years. All the patients reported working 9 h daily in the plant without personal protection garment (goggles, gloves and masks). Unfortunately, permission to visit the plant could not be obtained. All the information regarding the work conditions was provided by the patients. Information about work areas was available for 11 patients; seven of them (64%) worked in the killing area, whereas the remaining worked in animal cleaning and transportation. Two patients required hospitalization due to severe illness, whereas other 15 were outpatients. The first medical consultation of all the patients took place between January 2014 and July 2015.

The time elapsed from initial symptoms to diagnosis ranged from 2 to 60 days (median 17.7 days). Clinical data were available in 16 cases. As shown in Table 1, the most frequent clinical manifestations were fever, sweats, asthenia and myalgia.

Evidence for hepatic involvement was found in 13 of 17 patients. From 15 patients in whom liver enzymes were assessed, 11 (73.3%) exhibited increased GPT levels and four also had increased alkaline phosphatase. Abdominal ultrasound, performed in nine patients, yielded abnormal findings in six (hepatomegaly in four, splenomegaly in three, steatohepatitis in two and perihepatic adenopathy in one). Two patients with hepatomegaly had normal levels of liver enzymes.

Serological studies for HIV were performed in 11 patients and for HBV and HCV in nine, yielding negative results. Alcohol consumption was referred by seven patients. Haematological studies revealed lymphocytosis in 26.6% of the patients, leukocytosis with neutrophilia in 13.3% and mild anaemia in 21.4%.

Table 1. Clinical manifestations in brucellosis patients

Signs and symptoms	n	%
Fever	16	100
Sweats	15	93.7
Myalgia	12	75
Asthenia	11	68.5
Headache	8	50
Arthralgia	7	43.7
Rachialgia	6	37.5
Adenopathies	5	31.2
Hepatomegaly	4	25
Splenomegaly	3	18.7
Diarrhoea	2	12.5
Productive cough	2	12.5
Orchitis	1	6.2

Chest radiography was performed in 13 patients, from whom only two had respiratory symptoms. Radiological abnormalities were found in four cases (30%), including lobar pneumonia in two cases (one with pleural effusion) and interstitial involvement in other two. These four patients were considered to have respiratory involvement. The two patients with radiological pneumonia presented productive cough. Data obtained from clinical records did not reveal that these patients had a different exposure to the source of infection as compared to patients without respiratory involvement. From the four patients with respiratory involvement, only two worked in the killing area. Patients with respiratory involvement had no underlying condition that may predispose to respiratory infection. Three patients denied tobacco exposure, and no data were available from the fourth.

In one patient presenting left orchiepididymitis ultrasonography revealed a hypo-echoic and hypervascularized focal lesion.

One patient with a history of advanced osteoarthritis and bilateral hip replacement presented pain and functional loss in the left leg, which remitted after a prolonged anti-microbial therapy. Although the clinical picture strongly suggested a prosthetic infection, image-guided biopsy could not be performed to confirm osteomyelitis.

Rose Bengal test and STA test (titres from 100 to 6400) were positive in all patients. CF test was performed in 14 cases and was positive (titre $\geq$ 10) in 13 of them. C-ELISA was performed in 14 cases and was positive in all but one. Blood cultures were positive for *Brucella* spp. in 14 of the 17 patients (82.3%). All isolates were identified as *B. suis* biovar 1. Patients with respiratory involvement were already under anti-microbial therapy when this condition was detected. Therefore, *Brucella* isolation from respiratory samples or pleural effusion was not attempted.

Combined anti-microbial therapy was prescribed in all cases, as detailed in Table 2. All patients received therapy for 6 weeks except for the patient with advanced osteoarthritis who received doxycycline plus rifampicin for 48 weeks as joint infection was suspected. Patients with respiratory involvement were treated with doxycycline plus aminoglycosides (three cases) or TMS/SMX plus rifampicin (one case). Febrile illness remitted in all patients between 2 and 9 days of therapy. No clinical side effects were reported. Clinical relapses were detected in four patients at 3, 4, 6 and 9 months after initial anti-microbial therapy. *B. suis* was isolated from blood in two of them. Relapsing cases were treated with doxycycline–rifampicin (three cases) and doxycycline–gentamicin (one case). None of the relapsing cases corresponded to patients with respiratory involvement.

Table 2. Initial anti-microbial therapy schemes prescribed

Anti-microbial therapy	n
Doxycycline 200 mg + rifampicin 600 mg	12
Doxycycline 200 mg + gentamicin 320 mg	3
Doxycycline 200 mg + streptomycin 1 g	1
Rifampicin 600 mg + TMP/SMX* 640/3200 mg	1

*TMP/SMX: Trimethoprim/sulfamethoxazole.

Discussion

This report describes 17 cases of brucellosis among workers of a pork processing plant along 18 months. Notably, *B. suis* could be recovered by blood culture in 14 cases, probably due to the acute nature of this occupational infection. In the remaining three cases, the occupational setting, positive *Brucella* serology and a negative history for other potential sources of infection strongly suggested a *B. suis* infection.

As reported elsewhere, *B. suis* infections are mainly occupational, usually affecting pig slaughterhouses and pork processing plants (Hendricks et al., 1962; Escobar et al., 2013). In these settings, infection is frequently acquired from contaminated aerosols, conjunctival splashes or direct bacterial entry through skin lesions. Therefore, use of goggles, masks and gloves is strongly recommended. However, adherence to these measures is not adequately controlled in most pork processing plants of Argentina. The high rate of *B. suis* infection among workers of this processing plant may be related not only to the lack of adequate personal protection measures but also to the high prevalence of swine brucellosis in Argentina (Samartino, 2002, Castro et al., 2006). In agreement with previous reports, most cases of human brucellosis in this plant occurred among workers from the killing area (Hendricks 1962; Trout et al., 1995). After this outbreak took place, local sanitary authorities were notified of the brucellosis cases, and recommendations for reducing exposure to *Brucella* were made.

In agreement with previous reports on *B. suis* infection (Guerrier et al., 2011), fever, sweats, asthenia and myalgia were common symptoms in our patients. The frequency of hepatomegaly, splenomegaly and adenopathies was also similar to that reported previously (Dean et al., 2012). The most frequent complications of human brucellosis, which can appear in different stages of the disease, are osteoarticular, haematological and hepatobiliary. Genitourinary and neurological involvements are reported less frequently (Pappas et al., 2006; Dean et al., 2012). In this series, hepatic involvement was observed in 13 patients (~76%), respiratory involvement in four (~25%) and orchiepididymitis in one (~6%). The high frequency of hepatic involvement in our series also agrees with previous reports (Dean et al., 2012).

Anti-microbial therapy was successful in most cases (77%). Clinical relapse occurred in four patients, and in two of them, *B. suis* could be isolated by blood culture. Three of the four patients having relapse had received oral therapy (doxycycline and rifampicin), which may have reduced their compliance. The frequency of clinical relapse is in line with previous reports (Franco et al., 2007).

Respiratory involvement has been reported in less than 20% of human brucellosis cases (Wallach et al., 1994; Pappas et al., 2003), even among laboratory staff highly exposed to contaminated aerosols (Staszkiewicz et al., 1991). In this series, however, it was observed in 30% of the patients in whom chest X-ray studies were performed. A relationship between *B. suis* infection and respiratory involvement in these patients is strongly suggested by several facts. *B. suis* was isolated by blood culture in the four patients with respiratory manifestations. In addition, clinical and epidemiological data did not reveal other potential causes for the pulmonary findings. Finally, respiratory clinical manifestations declined with anti-microbial therapy. The high frequency of respiratory involvement in our series makes necessary to consider brucellosis in the differential diagnosis of respiratory diseases in pork processing plant employees.

Acknowledgements

We are grateful to Gabriela Escobar from ANLIS-Malbrán for typing clinical isolates of *Brucella*.

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