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Human brucellosis at a pig slaughterhouse

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ABSTRACT

Seventeen workers in a pig slaughterhouse with signs and symptoms compatible with brucellosis were clinically examined at the outpatient service of different health institutions and studied by serological tests during the period 2005–2011. Eleven blood cultures were taken and six *Brucella suis* strains were isolated, three biovar 1 and three with atypical characteristics. In order to confirm that these cases had no common source, a variable number of tandem repeat (VNTR) analyses were performed on 5 of the 6 strains whose results showed substantial heterogeneity in the genotypes, thereby demonstrating that the immediate origin was not the same. Two hundred adult pigs admitted for slaughter at the plant were sampled by convenience and tested by buffered antigen plate test (BPAT), serum agglutination test (SAT) and 2-mercapto-ethanol test (MET). Seven of 62 males (11%) and 25/138 (18%) females tested positive. The study results contribute information on risk scenarios for packing plant workers and underscore the need to improve plant workers' education on appropriate containment measures and to actively screen animals for swine brucellosis.

1. Introduction

Porcine brucellosis has been detected and identified in Argentina since the 1940s; several studies have mainly focused on establishing the regional prevalence in areas where this animal species was relevant. Sixty-eight percent of the porcine population is found in the provinces of Buenos Aires, Córdoba and Santa Fe, and between 1960 and 1980 several surveys found 14.2–25% prevalence in different regions [1]. However, there is no formal program to monitor the disease [1]. *Brucella suis* biovar 1 and *B. suis* biovar 1 with atypical characteristics have been sporadically isolated, but a bacteriological diagnosis is difficult to make in animals because of the cost and the lack of facilities and trained personnel. The serological tests prescribed for international trade are indirect ELISA (IELISA), competitive ELISA (cELISA), fluorescence polarization assay, Rose Bengal test (RBT) and complement fixation test (CFT) [2]. cELISA, RBT and CFT were developed to diagnose individual pigs and to screen large numbers of sera. However, the National Animal Health Service (SENASA) [3] recommends buffered plate antigen test (BPAT), serum agglutination test (SAT) and 2-mercapto-ethanol test (MET) to monitor porcine brucellosis in the country.

Since 1994 the National Laboratories and Institutes of Health Administration Dr. C.G. Malbrán (ANLIS) have been working on the serological and bacteriological diagnosis of human brucellosis. The aim of this paper is to describe cases of workers from a pig slaughterhouse, with signs and symptoms compatible with brucellosis whose diagnoses were

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confirmed at ANLIS between January 2005 and January 2011, and to report the detection of anti-*Brucella* antibodies in the pig population entering the processing plant in one day. In order to confirm that the cases had no common origin, a variable number of tandem repeat (VNTR) analyses were performed [4]. The results of this study were partially presented at the 64th Brucellosis Research Conference, Buenos Aires, Argentina, September 21st–23rd, 2011.

2. Materials and methods

2.1. Human cases

2.1.1. Clinical description

This condition is characterized by acute or insidious onset of fever and one or more of the following: night sweats, arthralgia, headache, fatigue, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis or focal organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly) [5].

2.1.2. Case classification

Suspicious: A clinically compatible illness, epidemiologically linked to occupational work with animals, consumption of animal products, laboratory exposure, etc. *Probable:* Suspicious case with one positive screening test such as BPAT, RBT or rapid slide agglutination test. *Confirmed*: A clinically compatible illness with definitive laboratory evidence of *Brucella* infection: culture and identification of *Brucella* spp. from clinical specimens, blood cultures, bone marrow, biopsies, etc. or evidence of positive serological tests such as SAT, CFT, cELISA, IELISA, or epidemiologically linked to a confirmed case of human or animal brucellosis. *Case ruled out*: Two samples drawn with a 30 day interval lacking evidence of anti-*Brucella* antibodies [5].

2.2. Patients

The 17 patients (one woman) were clinically examined at the Infectology Service of different health care institutions and samples were obtained for serological tests; blood cultures were done in 11 of them. Fever, headache, asthenia, arthralgia, night sweats, low body weight, hepatosplenomegaly and back pain were the main signs and symptoms. Some reported fatigue, malaise, chills, vomiting and emotional irritability. They had been working in the plant for 1–9 years in slaughtering and butchering and in areas such as loading and unloading, gutting and taking samples for healthcare. None of them had a history of brucellosis.

2.3. Serological tests

The classical tests: BPAT, RBT, SAT, MET and CFT were run as previously described [6] with antigens prepared at ANLIS using the *Brucella abortus* 1119-3 strain. cELISA was run as per previous report. The antigen (S-LPS from *B. abortus* 1119-3) and the mouse monoclonal antibody (MAb) was standardized and supplied by the Brucellosis Centre of Expertise and OIE Reference Laboratory, Animal Diseases Research Institute (ADRI), Ontario, Canada. The goat anti-mouse immunoglobulin G (IgG) antibody conjugated to horseradish peroxidase was from Jackson Lab, PA, USA. The test sensitivity was 98.3% and the specificity 99.7% at a cut off value of I 28% [7].

2.4. Control sera

Positive and negative human reference sera were included in every classical test and cELISA plate as controls.

2.5. Bacteriological studies

Brucella organisms were isolated from human blood culture by inoculating 5 ml of blood into a liquid medium (Hemo *Brucella*-Britania, Argentina or BACTEC-Bio Merieux, France). At ANLIS samples were incubated at $36 \pm 1\,^{\circ}C$ in 5–10% CO_2 for 30 days and the isolated strains were studied by direct observation, acriflavine test and crystal violet colonies staining [8]. Those were subsequently identified and typed by classical methods including CO_2 requirement, agglutination pattern with monospecific anti-A, anti-M and anti-R sera, urease test, production of H_2S, growth on dyes, erythritol, streptomycin and penicillin sensitivity and lysis by Tb and R/C phages following the previously described procedures; typed *Brucella* strains of each species were included in all tests as controls [9].

2.6. Variable number of tandem repeats (VNTR) typing

VNTR typing was carried out essentially as described by Whatmore et al. [4] omitting to examine a single locus of the 21 initially described. Twenty VNTR loci were targeted for amplification and subsequent allele size determination by running amplicons on an ABI 3130xl genetic analyzer (Applied Biosystems, Life Technologies, CA). Fragment analyses and final allele designation were based on amplicon size and determined with GeneMapper® Software (Applied Biosystems, Life Technologies, CA). Relationships between isolates were examined by cluster analysis using the categorical coefficient and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) implemented in Bionumerics Version 5.1 (Applied Maths, Belgium). The profile of the type strain *B. suis* biovar 1, 1330 was included for comparison.

2.7. Therapy

Treatment schemes varied according to the facility where patients were treated and the characteristics of the disease (acute or chronic, and localization); however, most cases received 1 g of intramuscular (IM) streptomycin for 15 days, and 100 mg of oral doxycycline b.i.d. for 45 days [10].

2.8. Slaughterhouse information

The plant slaughters pigs, processes pork meat, and also produces pork sausages as well as fat, blood powder and plasma. An average of 30,000 pigs from the provinces of Buenos Aires, Santa Fe, Córdoba, San Luis, Entre Ríos and La