

# Brucellosis outbreak in a rural endemic region of Mexico – a comprehensive investigation

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## Keywords

*Brucella abortus*,  
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Brucellosis,  
Cheese,  
Outbreak,  
PCR,  
Zoonosis.

## Summary

Brucellosis is a worldwide zoonotic disease. Generally, humans can be infected by either the consumption of raw milk and fresh cheeses made from unpasteurised milk or by contact with infected animals, mainly in endemic regions. In this study, we investigated a brucellosis outbreak in State of Guanajuato, an endemic region of Mexico. Microbiological culture of human blood, raw milk from cows and goats, and fresh cheeses was performed to isolate *Brucella*. Identification of the bacteria was done by bacteriological procedures and by multiplex Bruce-ladder polymerase chain reaction (PCR). *Brucella melitensis* was isolated from patients, infected goats, and fresh goat cheeses; while *Brucella abortus* was isolated from cows. All patients had eaten fresh cheese, but no occupational exposure to animals was reported. The results of molecular typing did not show any *Brucella* vaccine strains. The isolation, identification, and molecular characterisation of *Brucella* spp. in both human brucellosis cases and infected animals are very important to identify the source of infection and to take control measures in endemic regions.

## Indagine su un focolaio di brucellosi in una regione endemica rurale del Messico

## Parole chiave

*Brucella abortus*,  
*Brucella melitensis*,  
Brucellosi,  
Focolaio,  
Formaggio,  
Reazione a catena della  
polimerasi (PCR),  
Zoonosi.

## Riassunto

La brucellosi è una zoonosi diffusa in tutto il mondo. L'uomo può infettarsi sia con il consumo di latte crudo e formaggi freschi prodotti con latte non trattato di animali infetti sia tramite il contatto con animali infetti, soprattutto nelle regioni endemiche. Questo studio è stato condotto su un focolaio di brucellosi riscontrato nello stato di Guanajuato, una regione endemica del Messico. L'isolamento di *Brucella* è stato effettuato su colture microbiologiche di sangue umano, latte crudo proveniente da bovine e capre e formaggi freschi. L'applicazione di procedure batteriologiche e reazione a catena della polimerasi (PCR) di tipo Bruce-ladder multiplex ha permesso l'individuazione del microrganismo. *Brucella melitensis* è stata isolata in campioni prelevati da pazienti, ovini infetti e formaggi di capra freschi. *Brucella abortus* è stata isolata in campioni bovini. Dall'indagine è risultato che tutti i pazienti in precedenza avevano consumato formaggio fresco e che non erano stati soggetti a esposizione agli animali per ragioni professionali. I risultati della tipizzazione molecolare non hanno evidenziato

ceppi vaccinali di *Brucella*. L'isolamento, l'identificazione e la caratterizzazione molecolare di *Brucella* spp. costituiscono passaggi importanti per identificare la fonte di infezione e per adottare adeguate misure di controllo della brucellosi nelle regioni endemiche.

## Introduction

Brucellosis is one of the most widespread zoonoses in the world. It is an important public health issue in the Mediterranean, Western Asia, some regions of Africa, and some Latin American countries such as Colombia, Venezuela, Mexico, Brazil, Peru, and Argentina (Pappas *et al.* 2006). This zoonotic disease is caused by different *Brucella* species, the most important for domestic animals and human health are *Brucella melitensis*, *Brucella abortus*, and *Brucella suis* (Godfroid *et al.* 2005). Brucellosis is considered a re-emerging foodborne disease transmitted to humans mainly through the consumption of fresh cheeses made from unpasteurised milk, mostly raw milk in developing countries (Vassalos *et al.* 2009). Mexico remains a country where brucellosis is endemic, although a National Campaign for the eradication of animal brucellosis has been on-going since 1996. Most of the studies in Mexico are focused on describing the prevalence of brucellosis in humans and animals; less documentation is available on isolation and identification of the *Brucella* species (Oseguera *et al.* 2013). In endemic regions, the isolation, identification, and molecular characterisation of *Brucella* spp. in humans, animals, and dairy products need to be undertaken to identify the source of infection and plan appropriate control measures (Godfroid *et al.* 2013). In this work, we describe a brucellosis outbreak in a rural endemic region of Mexico where cattle is raised along with goats.

## Materials and methods

### Study area

The current study was conducted in a community located in the State of Guanajuato, Mexico. The climate of this region is generally warm throughout the year, with an average temperature of 18°C and a rainy period during Summer. The main economic activities are agriculture and livestock production, mainly of cattle and goats. Also, production of fresh goat cheeses using unpasteurised milk is common in this area. The protocols for humans were approved and conducted by the Escuela Nacional de Ciencias Biológicas, Institutional Animal Care and Ethics Committee<sup>1</sup>.

The State of Guanajuato is one of the first areas in which human brucellosis cases have been reported in Mexico. As the diagnosis of brucellosis is based on serological tests, no information about the *Brucella* species causing the infection in humans is available. At the beginning of 2012, some human cases were reported in a community of this region (with 10,324 inhabitants) prompting our study in this area with the aim of identifying the *Brucella* species in human cases, animals, and cheeses (Oseguera *et al.* 2013).

### Human cases

Fourteen brucellosis patients agreed to undergo serological and microbiological brucellosis tests through the Public Health Institute. Each patient signed a written informed consent; in the case of minors, the consent form was signed by their parents. Blood samples were cultured in modified biphasic Ruiz-Castañeda medium commercially available (INDRE-SSA, Mexico D.F., Mexico); sera were tested using the Rose Bengal test, the serum agglutination test, and agglutination with 2-mercaptoethanol<sup>2</sup>. A questionnaire-based survey was circulated among the patients.

### Animals tested

In this community, 700 cows and 336 goats were recorded. Out of 14 farmers, just 2 used the *B. abortus* S19 vaccine; twelve did not apply any vaccine. Most of the farmers were afraid to take part in this study, with the assumption that brucellosis-positive animals would be eliminated. Therefore, just 7 farmers agreed to take part in this study. The herds that were recently vaccinated with *B. abortus* S19 were excluded. We tested all cows from the 7 herds (3 herds had reported abortions recently), 300 cows in total. In the case of goats, the vaccine *B. melitensis* Rev 1 was not applied in this community. Owners were testing (serologically) and culling infected goats, recently abortions had also been recorded. All 336 goats were serologically tested. The blood samples were sent to the Official Animal Laboratory of Guanajuato (Mexico) for diagnostic tests. Sera from cows were analysed by

<sup>1</sup> Approved protocol number MIC/ENCB/4265/2011.

<sup>2</sup> Institute of Public Health SSA. NOM-022, Norma Oficial Mexicana 022, 2012. Para la prevención y control de la brucellosis en el hombre, en el primer nivel de atención. Diario Oficial de la Federación. (DOF: 11/07/2012NOM-022-2012-Brucellosis humana/ accessed on 27 January 2014).

the card agglutination test, and positive results were confirmed by means of Rivanol tests (agglutination titres  $\geq$  1:50 were considered positive for brucellosis) (World Organization for Animal Health 2013a)<sup>3</sup>. Sera from goats were analysed by the card agglutination test and positive results were confirmed by means of the complement fixation test (titres  $\geq$  1:8 were considered positive for brucellosis) (World Organization for Animal Health 2013b). Milk from positive cows and goats were taken for *Brucella* isolation. Also, fresh cheese samples were collected from the main producer from which the patients had bought cheeses.

### Isolation of *Brucella*

Briefly, 40 mL of milk sample was centrifuged at 4,000 rpm. The sediment and cream were spread on Farrell's agar plates and incubated for 15 days at 37°C in 5% CO<sub>2</sub> (World Organization for Animal Health 2013a). Five cow cheeses and 5 fresh goat cheeses were collected. Twenty-five grams of each cheese sample were homogenised using a Stomacher and cultured in an enrichment medium containing antibiotics (Oxoid *Brucella* supplement). After 72 hours, samples were cultured on Farrell's agar and incubated at 37°C in 5% CO<sub>2</sub>.

### *Brucella* identification

Colonies with a *Brucella* phenotype were tested by Gram stain, oxidase, catalase, and urease production, fuchsine and thionin sensitivity, phages, and monospecific serum (Alton *et al.* 1998). Also, *Brucella* strains were typed by the Bruce-ladder multiplex polymerase chain reaction (PCR) using 8 pairs of oligonucleotides, as previously described (García-Yoldi *et al.* 2006). We used the following *Brucella* type strains: *B. abortus* ATCC 23448 (544); *B. melitensis* ATCC 23456 (16M); and *B. suis* ATCC 23444 (1330). DNA was extracted from heat-killed bacteria using the modified Wilson's method (Wilson 1987). The concentration and purity of DNA were determined by spectrophotometric readings at 260 and 280 nm. The amplified products were resolved by electrophoresis using a 1.5% agarose gel, followed by staining with ethidium bromide.

### Results

All of the 14 patients were found to be positive by Rose Bengal, which was later confirmed by

agglutination tests. All patients received specific brucellosis treatment (rifampicin combined with trimethoprim/sulfamethoxazole for 42 days). *Brucella melitensis* biovar 1 was isolated from 2 patients: a 14-year-old teenager and a 44-year-old male. The survey showed no recent or earlier occupational exposure to animals in the human cases, but all patients reported eating fresh goat cheese bought from the same place. Most of the patients had taken antibiotics previously, as they were diagnosed with other febrile infections.

Milk from 40 serologically positive cows was submitted to *Brucella* isolation and 9 isolates of *B. abortus* biovar 1 were obtained. Also, 4 isolates of *B. melitensis* were obtained from 34 samples of raw milk from seropositive goats. We also isolated 2 strains of *B. melitensis* biovar 1 from 5 fresh goat cheeses.

The results of the Bruce-ladder multiplex PCR matched the microbiological typing of the *Brucella* isolates and no vaccine strains were found. Figure 1 shows the amplification products of *Brucella* isolates. DNA purified from *Brucella* type strains was included as a positive control.

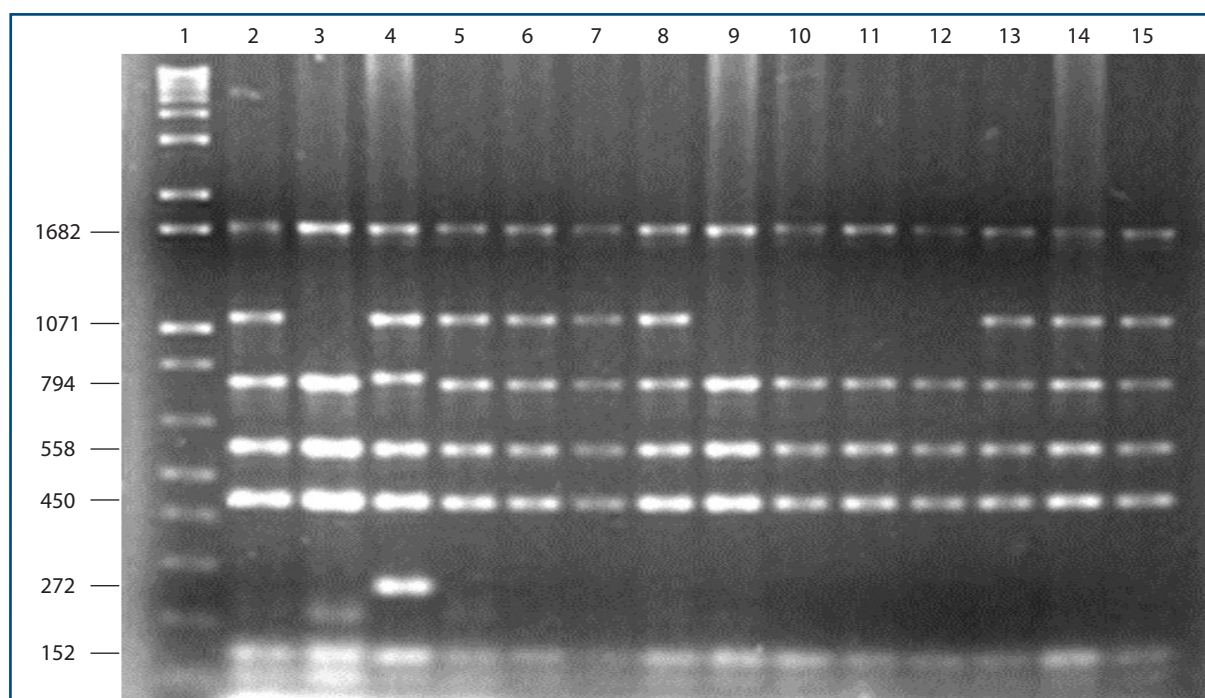
### Discussion

Despite government efforts to eradicate brucellosis in Mexico, some regions still have a high prevalence of animal brucellosis (Oseguera *et al.* 2013). In this work, we identified the *Brucella* species in a community located in an endemic brucellosis region of Guanajuato State. In 2008, this state reported a bovine brucellosis prevalence of around 4.1%, while the prevalence in goats was 2.86%<sup>4</sup>. A total of 2,531 human brucellosis cases were reported in Guanajuato in the last 5 years, but no reports of the *Brucella* species involved in those cases are available<sup>5</sup>. The isolation of *Brucella* is not a common practice in Mexico and has been performed only in some epidemiological investigations, focusing mainly on dairy products (Luna-Martínez and Mejía-Terán 2002). In the community tested in this article, we report the isolation of *B. abortus* in cows, *B. melitensis* in patients, and *B. melitensis* in cheeses. As the patients were infected by eating cheeses around half a month before we arrived in the community, we could not ensure that the tested cheeses were the same of those the patients had bought. We confirmed that the cheeses made by the main producer in that community contained *B.*

<sup>3</sup> Institute of Animal Health SAGARPA. NOM-041 Norma Oficial Mexicana, 1995. Campaña nacional contra la brucelosis en los animales. Diario Oficial de la Federación, 18 de julio de 1996. (Available: 08-20-96 NOM-041- ZOO-1995 brucelosis/ accessed on 27 January 2014).

<sup>4</sup> UNAM. Unidad de Posgrado de la Facultad de Medicina Veterinaria. Consejo Técnico Consultivo Nacional de Sanidad Animal. Plan Estratégico de la Campaña Nacional Contra la Brucelosis en los Animales 2008-2012.

<sup>5</sup> SUIVE, Sistema Único de Información para la Vigilancia Epidemiológica/ Dirección General de Epidemiología. Boletín epidemiológico. Cuadro 8 Brucelosis.



**Figure 1.** Bruce-ladder multiplex PCR profile of type *Brucella* strains and *Brucella* isolates from human blood samples, cow and goat raw milk samples, and fresh cheeses collected in the State of Guanajuato Mexico. 1) Ladder (1 kb); 2) *Brucella melitensis* 16M; 3) *B. abortus* 544; 4) *B. suis* 1330; 5-8) *B. melitensis* isolates from goat milk; 9-12) *B. abortus* isolates from cow milk; 13-14) *B. melitensis* isolates from patients; 15) *B. melitensis* isolate from goat cheese.

*melitensis*. We also found that the cheese producer used milk from goat flocks that were seropositive for brucellosis. Human brucellosis is caused mainly by the consumption of raw milk and fresh goat cheese, which is a traditional custom in the rural population, as it has been reported in Iran, Macedonia, Greece, and Argentina (Akbarmehr 2011, Bosilkovski *et al.* 2007, Karagiannis *et al.* 2012, Lucero *et al.* 2010).

Although in the study area raising cattle and goats is common, our results did not show cross infection, *i.e.* no *B. melitensis* was found in cows and no *B. abortus* in goats. Slaughtering of infected animals is one of the principal measures to control brucellosis (Marianelli *et al.* 2007, Alvarez *et al.* 2011). However, in developing countries where the government cannot pay compensation for infected animals because of a lack of economic resources, following through on the slaughtering is particularly difficult, since the animals are the sole source of income for many households. We found that the farmers in the community involved in this study did not slaughter infected goats, and they continued manufacturing fresh cheeses with unpasteurised goat milk. Recently, Mendez-Gonzalez and colleagues (Méndez-González *et al.* 2011) demonstrated that *B. melitensis* could survive the manufacturing process of 'Mexican style' fresh goat cheese. In Mexico, it is mandatory to pasteurise milk used for sale or for cheese production, but this does not prevent the production and sale of unpasteurised dairy products, especially in rural regions. In

brucellosis-free countries, the risk of acquiring brucellosis is through emigration or by travelling to endemic regions (Memish and Balkhy 2004).

In this work, we included the use of the Bruce-ladder, a multiplex PCR, which is able to differentiate *Brucella* species and vaccine strains: *B. abortus* S19 and RB51 and *B. melitensis* Rev 1 (García-Yoldi *et al.* 2006). We did not find vaccine strains in any *Brucella* isolate. We observed that only some owners applied the vaccine *B. abortus* S19 in cattle. It is worth stressing that 3 farmers who had goats seropositive for brucellosis had recently bought goats from traders without any certification of brucellosis-free status. Also, we found that in the positive herds seropositive animals were not removed. Oseguera-Montiel and colleagues (Oseguera *et al.* 2013) reported that the prevalence of animals positive for brucellosis was much higher in goats from the area where the brucellosis control campaign was negligible compared with the animals where the brucellosis campaign had been conducted for about 6 years.

Bruce-ladder multiplex PCR has been used to identify *Brucella* isolates in different countries (Nagalingam *et al.* 2012, López-Goñi *et al.* 2008). Other typing methods for epidemiological surveillance of brucellosis have been developed, like the hyper-variable octameric oligonucleotide fingerprint (HOOF) technique based on variable number tandem repeats (VNTRs) (Whatmore *et al.* 2006).

After this outbreak in Guanajuato, measures for brucellosis control were adopted in the community, such as maintaining strict control regarding the handling of pregnant animals, disinfection of all material in contact with infected goats, and surveillance of animals. However, seropositive animals are still moving between herds without sanitary control, at the same time massive vaccination is not yet performed in all rural communities, especially in the case of small flocks. Under these conditions, the spread of *Brucella* spp. from infected herds to non-infected susceptible herds may continue (Solorio-Rivera *et al.* 2007).

We point out that brucellosis control measures, besides being efficient, should also be realistic in developing countries where financial resources are scarce. We also stress the importance of identifying the *Brucella* species in endemic regions.

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