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Retrospective Brucellosis´ Study in Mexico

Review Article

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Author Details

*Rodríguez Pérez O[1], Ramírez Mata A[2], Galicia Domínguez JA[1], Ortiz González S[1], Báez Castillo E[3], Reyes Carmona SR[2] and Claudia Mancilla Simbro C[1,4]**

[1]*Facultad de Medicina Veterinaria y Zootecnia-Benemérita Universidad Autónoma de Puebla, Carretera. Tecamachal co-Cañada Morelos Km. 7.5, El Salado, Tecamachalco, Puebla. México*

[2]*Lab. Interacción patógeno-planta de Investigaciones -Centro de Ciencias Microbiológicas (ICUAP) Instituto de Ciencias-Benemérita Universidad Autónoma de Puebla. Puebla, México*

[3]*Facultad de Ingeniería. Benemérita Universidad Autónoma de Puebla. Puebla, México*

[1,4]*Lab. Fisiología y Biología Molecular de Células Excitables - Instituto de Fisiología. Benemérita Universidad Autónoma de Puebla. Puebla, México*

*Corresponding author

Claudia Mancilla-Simbro C, Laboratorio de Fisiología y Biología Molecular de Células Excitables - Instituto de Fisiología. Benemérita Universidad Autónoma de Puebla. Puebla, México

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Abstract

Background and objective: Brucellosis is a zoonotic disease transmitted from animals to humans. This disease has a high impact on the world and Mexico is one of the countries that today continues a campaign for its control and prevention. This disease is of bacterial origin: *Brucella spp*.; this has characteristics that make it different from others; its resistance to the environment, to changes in pH and to limitations in terms of nutrients. Another factor that generates interest is its invasion form and therefore its pathogenesis, preference to replicate in macrophages and releasing essential factors for their survival and multiplication.

Material and methods: a systematic review and meta-analysis of *Brucella abortus* from Puebla Mexico "Disease limited to one or several areas" was carried out, for *B. abortus* and *B. melitensis* in production animals (SENASICA, 2020; National Epidemiological Surveillance System, 2022). The data was compiled from 2020-2022, from the state of Puebla Mexico.

Results: Currently, more research continues on the virulence factors of the bacteria, finding new elements of studies in order to find a possible vaccine for the disease. There are tests such as the rose bengal test that help diagnose the disease for both animals and humans, although the options are diverse both in the field and in the laboratory.

Discussion and Conclusion: Today only prevention campaigns can be carried out in Mexico such as the S19 or RB51 vaccine, which is why treatment in humans is complicated given that several prolonged regimens are recommended, so prevention and good livestock practices they are the first line of defense to confront this disease.

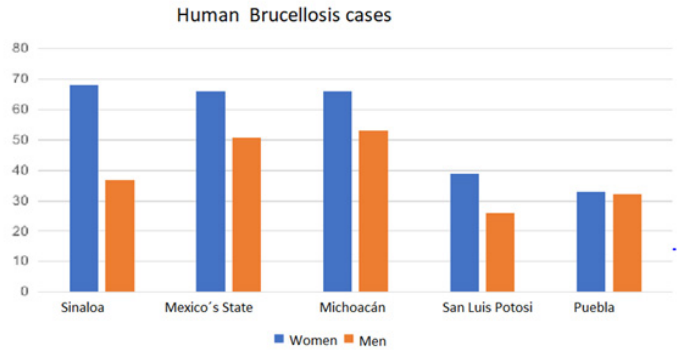
Background: Brucellosis is a disease transmitted from animals to humans, also known as zoonotic. This is the disease of its type with the greatest impact in the world and Mexico is one of the countries that continues today in the campaign for its control and prevention. The *Brucella* bacterium has characteristics that make it different from the rest, for example its resistance to the environment, to changes in pH and a limitation in terms of nutrients. Another factor that makes *Brucella* of great interest is the form of pathogenesis, preferring to replicate in macrophages and releasing essential factors for their survival and multiplication. Currently, more research on the virulence factors of the bacteria continues, finding new elements of studies in order to find a possible vaccine for the disease. There are tests such as the rose bengal test that help us diagnose the disease for both animals and humans, although the options are diverse both in the field and in the laboratory. Today only prevention operations can be carried out in Mexico such as the S19 or RB51 vaccine. Treatment in humans is simple since several prolonged schemes are recommended, so prevention and good livestock practices are the first line of defense to face this disease.

Keywords: *Brucella*, Brucellosis, Bacteria, Zoonosis, Virulence, Diagnosis, Strains

Introduction

Brucellosis is a bacterial disease caused by several species of *Brucella*, mainly affecting cattle, pigs, goats, sheep and dogs. Also capable of affecting humans; Contagion occurs through direct contact with animals, by eating or drinking products from sick animals or by inhaling airborne agents (WHO, 2020; 2023). Likewise, it is a zoonosis that implies a major public health problem and economic importance in countries that are considered endemic. The great diversity of animals that are carriers, as well as the multiple vectors that collaborate with its spread, complicate prevention actions; Even today there is no real overview of its prevalence. Furthermore, carrier animals are in intimate contact with humans, which increases the relevance and dimension of this health problem [1] The current situation of the disease places it as one of the most important zoonoses worldwide. It is a notifiable disease, so any event in a country, zone or compartment must be declared even in the absence of clinical signs, as established by the World Organization for Animal Health. Mexico has the status before the World Organization for Animal Health (OIE) of "Disease limited to one or more areas", for *B. abortus* and *B. melitensis* in production animals [2].

At the state of Puebla, 65 cases have been reported in 2020, of which 33 were male patients and 32 were female patients [2] (Graph 1). By the end of 2022, epidemiological surveillance of brucellosis cases showed a total of 101 cases, with 50 male patients and 51 female patients (National Epidemiological Surveillance System, 2022) (Table 1).



Graph 1: Situation of brucellosis in human patients. The scheme represents the states with the highest number of cases during 2020, according to SENASICA (2020).

Table 1: Cases of human brucellosis by state.

Cases of Human Brucellosis by State. Data Collected Until Week 51 from 2022. SINAVE (2022)		
State	**Number of cases (Men and Women)**	**Total**
Guerrero	M:72 W:53	125
Sinaloa	M:23 W:84	107
Puebla	M:50 W:51	101
Nuevo León	M:38 W:54	92
Michoacán	M:37 W:53	90

Etiology

Brucella is a genus of bacteria belonging to the phylum of Proteobacteria, within this phylum, there is the class Alpha proteobacteria, which encompasses bacteria with different shapes, lifestyles, metabolic capacities and ecological variation. From a lifestyle perspective, this class includes free-living, commensal, endosymbiont, opportunistic, and intracellular pathogenic bacteria [3]. *Brucella* is a genus of small gram-negative bacilli, 0.5-0.7μm in diameter by 0.5-1.5μm in length, with a predominance of short coccobacillary forms. They are immobile and strictly aerobic, slow growing and do not have a capsule neither form spores [1]. The genomes of all *Brucella* species have a similar size and genome atlas with an average genome size of approximately 3.29 Mb consisting of two circular chromosomes. Chromosome I is approximately 2.11Mb and chromosome II is approximately 1.18 Mb. The G+C content of chromosome I is 57.2% and that of chromosome II is 57.3% [4]. *Brucella* is an intracellular pathogen, during an infection it survives and multiplies in macrophages; bacteria adapt to acidic pH, low oxygen levels and low nutrient levels [5]. *Brucella* can survive freezing and thawing, but is susceptible to most common disinfectants. The bacteria remain viable in the environment for months, especially in cool and humid conditions [4].

Brucella Strains

In animals there are six identified species: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis* and *B. neotomae*; These strains are the main ones that affect humans. Unidentified *Brucella* species have been found in marine mammals. The proposed formal names for marine mammal strains are *B. maris* for all strains, or *B. pinnipediae* for pinniped strains (seals, elephant seals, and walruses) and *B. cetaceae* for cetacean strains (whales, porpoises, and dolphins). Some *Brucella* species include biovars. Five biotypes have been reported for *B. suis*, 3 for B. melitensis, and up to 9 for *B. abortus* (Table 2) [6].

Table 2: Recognized Brucella strains and their biovars (The center of Food and Security, 2010).

Recognized Strains of Brucella and their Biovars		
Species	**Known Hosts**	**Biovarieties**
B. melitensis	Goats, cattle, sheep, canids, man.	1-3
B. abortus	Bovines, canids, men	1-9
B. suis	Pigs, canids, man	1-5
B. canis	Canids, man	
B. ovis	Sheep	
B. neotomae	Rodents	
B. ceti	Dolphins, porpoises, whales	
B. pinnipedialis	Seals	
B. microti	Red foxes, field rodents	
B. inopinata	Unknown	

Transmission

Brucella organisms are found in highest concentration in the uterus of pregnant animals. Aborted fetuses, placental membranes and uterine secretions act as the main source of infection. Organisms shed in the milk of infected animals can transmit the infection to the newborn. Animals become infected by ingesting contaminated food and water or coming into contact with aborted fetuses, fetal membranes, and uterine secretions [4]. Common sources of infection in people include contact with animal abortion products; ingestion of unpasteurized dairy products, ingestion of raw meat or other undercooked meat products; contact with laboratory cultures and tissue samples and accidental injection of attenuated brucellosis vaccines [6].

Clinical Signs

Brucellosis generally begins as an acute febrile illness with nonspecific, flu-like signs such as fever, headache, malaise, back pain, myalgia, and generalized aches. Extreme sweating may occur, especially at night. Splenomegaly, hepatomegaly, cough and chest pain of pleural origin are sometimes observed. Gastrointestinal signs including anorexia, nausea, vomiting, diarrhea, and constipation occur frequently in adults and less frequently in children.

Generally in animals, it is usually a mild disease that may present the following characteristic signs:

- Reproductive disorders: infertility, abortion, retained placenta, neonatal mortality or weakness of offspring; the infected female shows few clinical signs until she aborts.
- Orchitis and epididymitis in males
- Arthritis, since occasionally the bacteria settles in the joints

Pathogenesis

Brucella strains have a strong tissue tropism and they replicate within the vacuoles of macrophages, dendritic cells and trophoblasts. However, the pathogen has the ability to replicate in a wide variety of mammalian cell types, including microglia, fibroblasts, epithelial cells, and endothelial cells [5]. *Brucella* moves through the mucos´ epithelial cell layer, where professional phagocytes (macrophages and DC cells) engulf the bacteria. The bacteria induce a zipper-like mechanism for internalization. Non-opsonized *Brucella* organisms are internalized through lectin or fibronectin receptors, but are opsonized through complement receptors. Opsonized bacteria are more likely to be destroyed within macrophages than non-opsonized ones. The pathogen binds to receptors containing sulfated residues and sialic acid on the surfaces of epithelial cells [7]. *Brucella* replication occurs after 12 hours, during which time mononuclear phagocytic cells trigger extensive transcriptional changes, including inhibition of apoptosis, prevention of DC maturation, reduction of antigen presentation, and reduction of of the activation of naïve T cells. Once adapted to the intramacrophage environment, *Brucella* extends its intracellular persistence indefinitely, contributing to systemic metastasis and infection of preferred target cells or tissues, such as placental trophoblasts, fetal lung, male genitalia, skeletal tissues, reticuloendothelial system, and endothelium [7].

Virulence Factors

Brucella does not produce classic pathogenic factors, such as: exotoxins, cytolysins, exoenzymes, exoproteins, capsules, plasmids, fimbriae and drug-resistant forms [8]. *Brucella* uses different strategies to invade its host cells.

Lipopolysaccharide

Lipopolysaccharide (LPS) is an essential virulence factor of *Brucella*. This is a constituent of the outer membrane of Gram-negative bacteria [9]. LPS consists of A lipid, oligosaccharide core and O antigen in gram-negative bacteria. Lipopolysaccharide is different and non-classical in *Brucella* compared to other gram-negative bacteria [5]. In *Brucella spp*., A lipid consists of a carbon structure of glucosaminyl-β (1-6) glucosamine substituted with saturated fatty acids. The central oligosaccharide contains glucose and mannose and is linked to A lipid via 2-keto-deoxyoctonate (KDO). The O antigen is formed by the polysaccharide α-1,2 or α-1,3 4-formamido-4,6-dideoxy-D-mannose [9] The importance of the O chain for the virulence of natural strains of *Brucella* lisa is well documented; One way in which the O LPS chain contributes to virulence is by protecting mild *Brucella* strains from the bactericidal activities of complement and antimicrobial peptides that they encounter during their interactions with host phagocytes [10]. Strains with smooth LPS are able to restrict host cell apoptosis through the interaction of the O chain with TNF-α (tumor necrosis factor). Therefore, dead cells do not release specific factors, therefore they do not activate the immune system and *Brucella* can avoid host immune surveillance [5]. The absence of the O chain of LPS defines the rugose phenotype and has been associated with an attenuation of virulence for most *Brucella* genera [11]. However, *B. ovis* and *B. canis* strains are naturally rugose and still virulent, indicating that other factors influence bacterial virulence [9].

Two-Component Regulatory System BvrR and BvrS

The two-component BvrR-BvrS system from *Brucella spp*., consists of a regulatory histidine kinase protein and a sensor protein, called BvrR and BvrS respectively [9]. BvrR directly controls the expression of omp25, omp22, and genes involved in A modification lipid of LPS

[10]. These proteins affect the transcription of membrane proteins: Omp3b (Omp22) or Omp3a (Omp25a) and have the influence on other non-protein membrane molecules and, therefore, on the functional and structural homeostasis of the membrane. The action of the BvrR/BvrS regulatory system activates the sensor domain of the BvrS protein by environmental signals through kinase activity. Additionally, BvrS causes phosphorylation and activation of the BvrR protein. BvrR activates transcription of omp3a, omp3b and other genes responsible for A lipid structure and perhaps the LPS core. Consequently, BvrS/BvrR mutants are more sensitive to cationic peptides and show increased permeability for surfactants [5].

Cyclic β 1,2 Glucans (CβG)

Cyclic glycans are present almost exclusively in members of the α-2-proteobacteria group, such as Agrobacterium and Rhizobium, and are involved in the interaction of the bacteria with the host organism [9]. Likewise, *Brucella spp*. and o secretes a cyclic polymer consisting of 17 to 20 glucose residues into its periplasmic space [10]. These glycans participate in the control of phagosome-lysosome fusion. The mutants are destroyed in phagolysosome and are not able to multiply. Even more, mutants treated by CβG are able to control vacuole maturation and lysosome fusion, so they can reach the ER and replicate there [5]. CβG has also been shown to have a strong influence on the ability of macrophages and dendritic cells to produce pro- and anti-inflammatory cytokines [10].

Superoxide Dismutase (SOD)

Macrophages have the ability to respond to the presence of *Brucella* by preventing cell replication, through a primary mechanism of bacteria destruction called Reactive Oxygen Intermediates (ROI) of which we find superoxide, hydrogen peroxide and hydroxyl radical. SOD is made up of iron and magnesium or zinc and copper, they are metalloenzymes responsible for the decrease in oxygen reactants. Some *Brucella* strains such as *B. abortus, B. melitensis* and *B. suis* possess two types of SOD. The first of them is SodA made up of manganese and this neutralizes the endogenously generated O. The second is SodC composed of copper and zinc responsible for neutralizing exogenously generated O and protecting against respiratory burst within macrophages [5].

Urease

Urease is a metalloenzyme, which decomposes urea into carbonic acid and ammonium form, and results in an increase in pH. This characteristic allows its survival in an acidic environment [5]. Most *Brucella* strains produce urease, and this enzyme is thought to protect them from the extremely acidic conditions they encounter during passage through the gastrointestinal tract after ingestion [10]. On chromosome I, there are two urea operons: ure-1 and ure-2; Ure-1 and ure-2 encode structural genes: ureA, ureB, ureC and accessory genes: ureD, ureE, ureF and ureG. Of the documented strains *Brucella ovis* is an exception, and it has been proposed that the absence of urease activity in this bacterium is one of the reasons for its limited host range and lack of oral transmission [5].

Outer Membrane Proteins (OMP)

The major outer membrane proteins (OMPs) of *Brucella spp*. were initially classified into three groups according to their mass: group 1 (88-94 KDa), group 2 (36-38 KDa) and group 3 (31-34 and 25-27 KDa) [9]. In the last group are highly conserved OMPs known as Omp25, Omp25b, Omp25c, Omp25d, Omp31, Omb31b and Omp22 that play an important role in maintaining the integrity of their cell envelope. These OMPs work in conjunction with the O LPS chain to protect these bacteria from complement and other antimicrobial peptides found in the host, and their contributions to virulence appear to be especially important for naturally occurring rugose strains such as *B. ovis*. The Omp25 protein from *B. abortus*, a smooth strain, also directly interacts with the SLAMF1 protein on the surface of dendritic cells and inhibits their maturation and ability to produce inflammatory cytokines [10].

Type IV Secretion System (T4SS)

The type IV secretion system (T4SS) is compiled by the virB operon, composed of 12 genes, which are believed to be essential for intracellular survival and share homology with other bacterial type IV secretion systems involved in trafficking. intracellular pathogens [11]. The T4SS *Brucella* consists of 11 proteins, 8 of which constitute the core of the transporter (VirB2, VirB3 and VirB5 to VirB10), 2 ATPases (VirB4 and VirB11) that provide energy to drive effector secretion, and a lytic transglycosylase (VirB1) which remodels the peptidoglycan layer of the bacterial cell during T4SS assembly [10]. A functional T4SS is required for the survival of *Brucella spp*. within macrophages and epithelial cells in vitro, as mutants lacking any of the virB genes do not replicate intracellularly [12].

One of the main functions of T4SS is to control the intracellular trafficking of *Brucella*-containing vacuoles in host macrophages in order to avoid death and degradation in phagolysomes. After entering the host cell, *Brucella* resides in a compartment called the endosomal *Brucella*-containing vacuole (eBVC), which will have limited interaction with the lysosomal pathway. These compartments serve as a signal for the induction of the genes that encode the T4SS. The intracellular trafficking of EBVC changes route to now make an interaction with the endoplasmic reticulum leading to the formation of replicative BVCs (RBVC) where *Brucella* maintain their chronic intercellular persistence. Subsequently, it will interact with the autophagy pathway of the host cell, generating an autophagic BVC that is believed to be important for bacterial egress and cell-to-cell propagation [13].

Diagnostic Tests

Humans

Presumptive Agglutination Test with Rose Bengal Antigen: indirect method that uses inactivated and stained *brucellae* that, by observing agglutination, demonstrates specific antibodies in the serum of the patient suspected of the disease. The interpretation of the result is qualitative (positive or negative), positive presence of agglutination, negative absence of agglutination. If the result is positive (presumptive test), it must be confirmed by SAT and 2-ME tests. Standard Agglutination Confirmatory Test (SAT): consists of the demonstration of anti-*Brucella* antibodies by agglutination, using inactivated bacteria that allow the identification of specific immunoglobulins of the classes IgM (demonstrates infection in the initial stage), IgG (demonstrates infection in the chronic stage) and IgA (demonstrates previous infection).

Confirmatory Agglutination Test in the Presence of 2-Mercaptoethanol (2-ME): for the demonstration of anti-*Brucella* antibodies by agglutination in the presence of this reagent, it is similar to the SAT test, but when 2-mercaptoethanol is added it inactivates IgM so if agglutination occurs these will be IgG [14].

Animals

Test card or Rose Bengal consists of comparing the problem serum with the *B. abortus* strain 1119-3 antigen at a concentration of 8% (8% Aba Test Card) for diagnosis in cattle and 3% in goats (3% Aba Test Card). . With this test, the presence of circulating IgG and IgM antibodies of vaccine origin or due to natural infections is detected. This test is routine and has a close sensitivity to 100%, meaning it will give results with few or no false negative animals.

The rivanol test is quantitative and qualitative; It consists of confronting the test serum with an acridine dye that precipitates the immunoglobulins of the sample, mainly the IgM, leaving only the IgG in solution, which are those directly involved with the immune re-

sponse to a field strain. It is then performed in a similar way to the plate agglutination test using a specific antigen. All sera that present a complete agglutination reaction in any of their dilutions are considered positive [15].

Prevention

Vaccines Available for Use in the Campaign Against Brucellosis in Mexico

Strain S19: Strain 19 is a live attenuated vaccine and the first *B. abortus* vaccine to be widely used for the control of bovine brucellosis. In the United States, this vaccine was used for more than five decades starting in 1941 and is still used in several other countries [16]. The vaccination campaign uses two modalities of the S19 vaccine: the first is known as the classic dose vaccine (Brucel N-19). The second is known as the reduced dose vaccine (Brucel R-19) which is applied to females over six months of age that did not receive vaccination with the classic dose, even if they are pregnant. Under no circumstances can the classic dose presentation vaccine be diluted to obtain the reduced dose vaccine [15].

It is used as a live vaccine and is normally administered to calves between 3 and 6 months of age as a single subcutaneous dose of 5-8 × 1010 viable organisms or as a reduced dose of 3 × 108 to 3 × 109 organisms which can be administered subcutaneously to the adult cattle. Alternatively, it can be administered to cattle of any age as one or two doses of 5 × 109 viable organisms, given conjunctively [17]. The S19 vaccine can provide better protection in adult cattle, prevent spontaneous abortion, reduce the epidemic in herds, but its protective effect depends on the age of inoculation, the dose, the immunization route and the immune status of the cattle [18].

Inoculation of cattle with strain 19 induces significant protection against abortions or infections caused by virulent strains of *B. abortus* and provides almost lifelong immunity against brucellosis. A major disadvantage is that vaccination of cattle with strain 19 induces serological responses that cannot be easily differentiated from responses induced by field strains of *B. abortus*. Although vaccination of calves between 4 and 12 months of age reduces the incidence, immune responses may persist into adulthood in a small percentage of beef calves. A second disadvantage is observations that strain 19, although less virulent than field strains, can induce arthritis in calf vaccinations [19].

RB51 vaccine: The RB51 strain has a rough morphology, as it lacks the "O" chain of the lipopolysaccharide. This characteristic gives it a certain advantage, since it does not induce the presence of antibodies that can be detected during sampling in official campaigns for the diagnosis of the disease; Therefore, it is possible to differentiate vaccinated animals from infected ones [15]. The RB51 vaccine formulation is the complete organism of *B. abortus* strain RB51. Adjuvants are not used in the RB51 vaccine. The approximate characteristic is due to the insertion of the IS711 element that disrupts the whoA gene encoding a glycol transferase in strain RB51. Due to the approximate characteristic, immunization of animals with RB51 can be easily differentiated from naturally infected animals, enabling effective vaccination policies [18].

In general, the recommended dose for RB51 vaccination is 1.0- 3.4 × 1010CFU. Protection against *B. abortus* infection is similar across the suggested dose, although higher antibody titers and longer bacterial persistence were associated with the full dose (3.4×1010CFU). Reduced dose (1×109CFU), generally recommended for adult animals, also protects against infection and abortion caused by virulent 2308. Although RB51 has very reduced abortive characteristics, it is not completely safe for pregnant cows, mainly when the full dose is administered [16].

However, although the RB51 strain has an excellent stability record, it is resistant to the important antibiotics rifampicin used in the treatment of brucellosis; Furthermore, it remains infectious to humans and the exact nature of its mutations has not been described. Recently, it was reported that RB51 vaccinated cattle in the Yellowstone metropolitan area of the United States were still susceptible to brucellosis [17]. Strain RB51 is cleared from lymphatic tissues more rapidly than strain 19 and, unlike strain 19, is not associated with significant lymphocytopenia. Vaccination of adult cattle with a reduced dose of strain RB51 (1x109 CFU) induces high protection against challenge with experimental *Brucella* in subsequent pregnancy. Although the 1x109 CFU dose has been shown to be safe for pregnant animals in field studies, abortions have been recorded after inadvertent administration of the full dose for the calf period during gestation [19,20].

Discussion

Brucellosis is a zoonotic disease that represents a major problem for public health and veterinary health. The disease has clinical signs that are important when making clinical diagnoses in both humans and animals, which facilitates the epidemiological monitoring of the disease. The characteristics of the bacteria are of interest since not having classic virulence factors makes it a pathogenic agent to which much more studies must be dedicated to determine how the bacteria acts within the host organisms. Among the documented virulence factors we find a broad list within which one factor in particular stands out: the type IV secretion system. This virulence factor has been established as responsible for triggering various actions that allow *Brucella abortus* to make its intracellular transit within the macrophage to complete its replication process. Although there are preventive vaccines, diagnoses often yield false positives precisely due to the use of the vaccine strain, which makes epidemiological monitoring difficult, not to mention that these vaccines are only intended for the livestock sector; at the moment there is no vaccine for humans.

Conclusion

Brucellosis is the disease that has had the greatest impact on livestock and human health, which is why it represents a great challenge to find an effective treatment for both groups, but today prevention can only be chosen in the animals, however, preventive practices and greater dissemination of the disease must be reinforced to be able to control the most important zoonosis worldwide.

Conflicts of Interest Statement

The authors have declare that no competing interests exist.

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