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Research Article

Evaluation of the Western Blot Technique for the Detection of Lipopolysaccharides and Glycoproteins from Specific *Brucella* Antigens in Northeastern Mexico

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Abstract

Brucellosis is a zoonosis of worldwide distribution, and an economically important infection of humans and livestock. The main species have been isolated from human infections are *B. abortus* and *B. melitensis*. The objective was to evaluate the Western blot technique for the detection of lipopolysaccharides and glycoproteins from specific *Brucella antigens* in northeastern Mexico. The study was descriptive and cross-sectional where 79 Goats of Saneen, Alpina, Toggenburg and Nubia breeds were sampled from a herd of the Ejido La Victoria in Mapimí, Durango, as a test sieve rose bengal was processed, the extraction of total proteins was also carried out membrane of the blood samples and thus the detection of Brucella by Western-blot. Of the total of 79 goats sampled from the herd of Ejido La Victoria in the municipality of Mapimí, Durango, 34 were positive to the Rose Bengal test used as a screening test, resulting in a seroprevalence of 43,03% In the present work it was evident the lipopolysaccharide exposure of B. melitensis evidenced by the serological reaction observed with the Rose Bengal antigen and with respect to the Western blot the protein of 37 kDa of both samples was detected by the sera of the samples RB positive of *B. melitensis*. By the Western blot method, 28 immunodominant proteins were detected in sera evaluated previously with the Rose Bengal test, it was observed that from the group of 4 goat breeds studied, the exclusive presence of a protein in a specific breed was not found, giving understood that *B. melitensis* does not discriminate between races. It is proposed that the 37 kDa protein is the most suitable to be used as a good marker of B. melitensis in goats and can be taken as reference for further studies in order to establish specific biomarkers for the diagnosis of this etiological agent.

Keywords: *Brucella*; Sensitivity; Specificity; Diagnosis

Introduction

Brucellosis is a highly infectious zoonotic disease of global significance caused by bacteria of the genus *Brucella* spp [1,2], it is an important re-emerging bacterial zoonosis that affects a wide range of different species of animals and humans throughout the world and is important from an socio-economic and public health point of view [3-7]. Due to abortion, *Brucella* infection is considered as one of the main factors that can cause massive economic losses in sheep and goats. Literature reveals that distribution of zoonotic *Brucella* species and their biovars vary with different geographical regions and husbandry practices [8]. Almost 500,000 new brucellosis cases are reported yearly in humans globally, with around 10/100,000 population [6,9]. Belonging to the genus *Brucella* facultative intracelular [6,9-11].

Brucellosis is a highly contagious bacterial disease of zoonotic importance that causes significant reproductive loss due to high abortion, birth of weak offspring, stillbirth, retained placenta, and

infertility rates in cows and ewes [4,6,12-14]. Direct contact with infected animals or their products, such as meat or milk, and contact with animals that have had a spontaneous abortion can be transferred [14,15]. This can result in acute febrile illness, commonly known as undulant fever. The diagnosis of brucellosis depends mainly on the epidemiological history of the patient, clinical manifestations, serological tests and bacterial cultures [9]. It is well known that individuals who work closely with animals are at a high risk of contracting the disease [14]. The *Brucella* genus includes 13 species infecting a range of susceptible host, but those of greatest one-health relevance include species infecting dogs (*B. canis*), swine (*B. suis*), cattle and domestic bison (*B. abortus*), and *B. melitensis* infects sheep and goats [2].

The main species that have been isolated in humans are *B. abortus* and *B. melitensis*, the latter being the most frequent cause of human brucellosis worldwide *B. abortus* [10]. The term brucellosis is applied to a group of infectious diseases caused by pathogenic gram-negative bacteria of the genus *Brucella* [6]. Members of the *Brucella* genus are transmitted from animals to people most frequently through occupational exposure or ingestion of unpasteurized dairy products, accidental contact with contaminated waste, for example inhalation of dust from dried excrement, which is the main source of acquisition of the bacteria causing a serious and debilitating disease that requires expensive and long-term treatment, which often leaves permanent sequelae, which can cause enormous economic losses in endemic regions, in animals domestic and considerable human morbidity [6].

B. melitensis, a species described in the genus *Brucella,* causes abortions in goats and sheep and Malta fever in humans. In humans, *B. melitensis* is highly pathogenic, making it one of the most serious zoonoses in the world. Brucellosis, also known as Mediterranean fever, Malta fever or wave fever, is a zoonotic allergic disease caused by *Brucella* that can trigger epidemics that seriously endanger the health of humans and animals [16]. Mediterranean fever is an extremely infectious zoonotic disease caused by *Brucella* spp, which is a Gramnegative bacteria that affects humans and animals and poses a serious threat to public health [6,17].

Brucellosis is transmitted to humans by direct contact with animals or consumption of infected unpasteurized or insufficiently cooked milk or dairy products [8,17], inhalation of infected aerosol particles and, to a lesser extent, meat from cattle, sheep, goats, pigs, camels, yaks, buffaloes or dogs.

Brucellosis presents a significant economic burden because it causes failures in the reproduction of the species itself and chronic health problems in humans. The bacteria has strong resistance in the natural environment and is sensitive to light, heat, acid, and common disinfectants. *Brucella* can enter the body through direct contact, conjunctival inoculation, gastrointestinal tract, respiratory tract, and biological transmission. While, nosocomial contagion is an important mode of infection [9].

Like other Gram (-) bacteria, *Brucella* expresses lipopolysaccharide (LPS), a major component of the outer membrane. The three structural components of LPS are lipid A, core oligosaccharide, and O antigen [18]. LPS, outer membrane protein (OMP), Type IV secretion system (T4SS), and BvrR/BvrS system contribute to virulence of *Brucella* [9].

Brucella vaccination is widely used for the prevention and control of brucellosis. But while the vaccines are considered sufficiently qualified for animal use, they can still be pathogenic for human use. Vaccination in reality remains the only rational strategy to confer protection to populations living in endemic countries. Efforts to develop an effective vaccine have been undermined by poor knowledge of the life cycle of *Brucella* in vivo and the protective immune response induced by infection.

In Mexico, the state of Sonora is the only one that is free of brucellosis due to smooth species in the Mexican Republic, 29,16% of the national territory is in the eradication phase (Campeche, Colima, Guerrero, Nayarit, Quintana Roo and Yucatán), while the States of Durango and Coahuila remain in a state of control [19].

The control of reproductive disorders in livestock depends on the systematic and coordinated efforts of the country's Veterinary Services at the national and regional levels and requires financial resources. Animal trade and transportation are factors that contribute to the spread of diseases [20]. The massive economic losses due to brucellosis in sheep and goats mandate the use of rapid and sensitive diagnostic techniques for appropriate detection, so as to determine and implement proper control strategies for the eradication of this disease [6]. An effective disease surveillance mechanism should be devised to determine the load of disease in livestock and human populations at regional and national levels for its efficient control [8]. According to SAGARPA (2023), the State of Baja California Sur is recognized as free of brucellosis, and So-

nora is free of brucellosis caused by smooth species, both entities make up 12.92% of the territory. 29.65% of the national territory is recognized in the eradication phase [21]. However, a large part of the northern states of Mexico have not been able to control animal brucelosis, and strengthening the animal health status has an impact on public health, since it mitigates the risk of contagion in humans due to the consumption of unpasteurized dairy products. Brucellosis is a cosmopolitan bacterial infection, being an endemic disease in Mexico and an important problem both nationally and worldwide. In our region, dairy production is of great importance to the economy. Due to this disease, production and quality are reduced and animals are lost due to reproductive failure. All of this is due to the lack of prevention and vaccination campaigns for livestock or animals. the poor knowledge of livestock farmers about the consequences that the disease can bring.

To date, the rose bengal serological test for the diagnosis of brucellosis has not been able to be replaced due to its great specificity, sensitivity, speed, and other demonstrable advantages for both the diagnosis of human and animal brucellosis. For some time now, work has been underway in the search for an antigenic component that is unique and specific to the *Brucella* genus and that on paper leads to the generation of a test that is equally easy to execute as those described until today. The development of the Western-blot technique allows, first, to extract the total proteins from the control strains, analyze their similarities in terms of protein components, subsequently identify the pattern of immunogenic proteins using the sera of the samples and thus, finally, evaluate whether There are differences between the patterns of immunogenic proteins between the samples under study. The objective was to evaluate the Western blot technique for the detection of lipopolysaccharides and glycoproteins from specific *Brucella* antigens in northeastern Mexico.

Material and Methods

The study was carried out at the facilities of the Faculty of Chemical Sciences of the State of Durango (UJED), Gómez Palacio Unit, Durango, in the Laboratory of Immunology and Gene Expression of the Faculty of Chemical Sciences of the University Juárez del Estado of Durango, Durango Unit and the Animal Anatomy Laboratory of the Regional University Unit of Arid Zones of the Autonomous University of Chapingo. The field work was carried out in the Ejido La Victoria of the municipality of Mapimí, Durango. The type of study was descriptive and cross-sectional. The study was descriptive and cross-sectional, approved by the Research Ethics Committee of the FCQ of the UJED N°123301538X0201 COFEPRIS. After the evaluation carried out, with an authorized opinion where 79 goats of Saneen, Alpina, Toggenburg and Nubia breeds were sampled from a herd of the Ejido La Victoria in Mapimí, Durango, where blood samples were collected from each goat with Vacutainer tubes without anticoagulant by puncture into the animal's jugular vein, each tube was labeled using codes that specified the animal's identification number, breed, and approximate age range. Subsequently, the samples were transported in coolers for centrifugation.

The blood sample was allowed to rest by placing the tube horizontally at room temperature until the clot formed. To avoid hemolysis, the clot must be separated, for which the tube was carefully uncapped, preventing the portion of clot attached to the stopper from detaching and breaking. In case a portion of clot breaks or remains in the serum, it was removed with long wooden applicators. The sample was placed in refrigeration for transport to the laboratory.

Serum was separated from blood by centrifugation at 3000 rpm for 20 min. Each sample was marked using codes describing each animal. Because isolation of *Brucella* from infected people or animals is difficult and prolonged, serological tests are used and relied upon for the routine diagnosis of brucellosis. Agglutination tests for diagnosis are the most used in Mexico, such as the rapid plate test with Rose Bengal antigen.

Rapid plate test with Rose Bengal antigen

The blood was centrifuged at 2,500 rpm for 10 min and the serum was separated from the cell package. Using a serological pipette, 30 µL of serum was taken from the sample and placed on a serological glass microplate. One drop of Rose Bengal antigen (*B. abortus* SAGARPA B-0653-023) was added to the 30 µL of serum in the serological plate. Using a wooden applicator, the serum and the Rose Bengal antigen were mixed in a circular manner for homogenization. The plate was shaken manually for 3 min. The plate was observed against an illuminated background and the results were recorded over time. The sample was reported as positive if agglutination occurred and negative if there was no reaction. The process was repeated for the 79 serum samples.

Biological material

For the development of the Western blot technique, the protein extract of the *B. melitensis* 16M strain provided by Dr. Maribel Cer-

vantes Flores of the Faculty of Chemical Sciences, Durango Unit, was used.

Electrophoresis and Western blot

A 10% polyacrylamide SDS-PAGE gel was prepared, with a separating gel (H_2O, 30% acrylamide, 1.5 M Tris pH = 8.8, 10% SDS, 10% ammonium persulfate and TEMED) and a concentrator (H_2O, 30% acrylamide, 1 M Tris-HCl pH=6.8, 10% SDS, 10% ammonium persulfate and TEMED). 20 µL of sample (5 µL of sample buffer [1M Tris-HCl pH=6.8, glycerol, SDS, β-mercaptoethanol, bromophenol blue and H_2O) were loaded into the electrophoresis equipment (Mini-Protean Tetra System Bio-RAD). distilled] 6.7 µL of tridistilled H_2O and 8.3 µL of the protein extract of the *B. melitensis* 16M strain) a loading buffer pH = 8.3 (Tris, glycine, SDS, distilled H_2O) and migrated at 100 v for 2 h, 5 µL of protein molecular weight marker were used for each gel (Precision Plus Protein TM Standards All Blue BIO-RAD®).

Subsequently, the transfer was carried out to a nitrocellulose membrane with the equipment (Trans-Blot SD Cell BIO-RAD) using a transfer buffer pH=8.3 (25mM Tris, 192 mM glycine, 20% methanol and distilled H_2O) at 15 volts for 40 min. Once the transfer was carried out, the membrane was washed 2 times for 3 min with 1X PBS pH=7.4 while stirring. The membrane was blocked by immersing it in a preparation of 5% Svelty milk in PBS Tween for 2 h. Three washes of PBS Tween were carried out again for 5 min while stirring.

Once the blocking was completed, the membrane was cut into strips for each sample and 1 mL of serum from each sample was added per strip. The sera were used as primary antibody in a 1:600 dilution with PBS Tween and the strips were allowed to incubate all the time. evening. The next day, the antibody was discarded and two washes were performed again with PBS Tween for 5 min while shaking. The addition of 1 mL of the secondary antibody (mouse anti-goat IgG conjugated with horseradish peroxidase) was continued with the addition of 1:1000 dilution with PBS Tween and left to incubate for 2 h with shaking. The secondary antibody was discarded again and washed again 2 times with PBS Tween while stirring for 5 min.

Finally, chemiluminescence development was carried out with the reagents (Clarity Western ECL BIO-RAD®), preparing 1 mL of the reagent (500 µL of peroxide solution and 500 µL of luminol solution), which was added to the membrane strips, covering it completely and waiting 5 min in darkness for the reaction to take place, then the excess reagent was removed and the membrane strips were taken to the photodocumentator (Chemi Doc XRS®) to capture the images.

Bioinformatic analysis

The bioinformatic analysis of the proteins that were revealed by chemiluminescence on the nitrocellulose membrane, as well as the determination of the detected bands and the corresponding molecular weight, were performed with the software (Image Lab 3.0) from Bio Rad®.

Results

Rose Bengal agglutination test

Of the total of 79 goats sampled from the Ejido La Victoria herd in the municipality of Mapimí, Durango, 34 were positive to the Rose Bengal test used as a screening test and 45 were negative to the same test, resulting in a seroprevalence of 43.03% as indicated in Table 1.

Total samples	Positive	Negative	Seroprevalence (%)
79	34	45	43.03

Table 1: Rose Bengal test results.

The Rose Bengal antigen reaction was considered positive when the presence of agglutination was observed in the reaction, and negative when there was no agglutination after 3 min.

Western blot

From the 79 samples collected that were processed with Bengal rose, the results were divided into positive and negative, subsequently electrophoresis and Western blot were performed where four gels were run with 26 positive and 10 negative samples for Bengal rose respectively. Chemiluminescence was used as a detection method, by reacting the membranes with luminol and peroxide solution, the Chemi Doc XRS photodocumenter captured the images corresponding to the four gels, the detection of the bands and the molecular weight was determined with the Image software. BiorAD Lab 3.0, where the results mentioned below were obtained. In gel number 1 (Figure 1), 10 positive samples were processed, showing proteins with molecular weights of 125, 95, 75, 62, 52, 44, 40, 37, 34, 28, 26, 21 and 14 kDa.

In gel number 2 (Figure 2), 8 samples were run, of which membrane 1, 2 and 3 are positive for Rose Bengal, showing proteins with

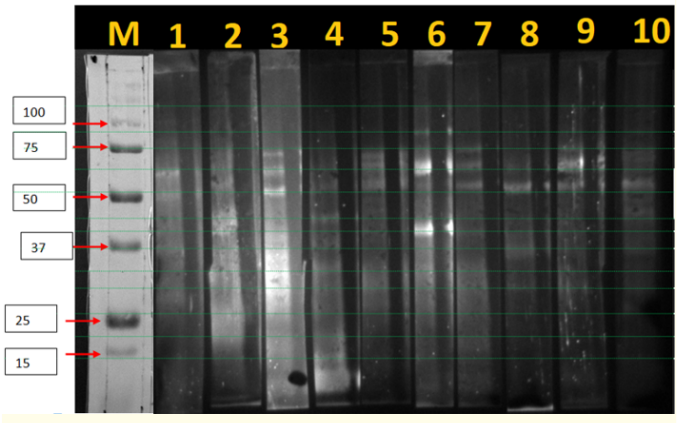

Figure 1: Representative Western blot of the proteins revealed by chemiluminescence of gel number 1. The molecular weight marker of 250 kDa is indicated with M, and serum samples 1-10 are positive for Rose Bengal.

molecular weights of 67, 52, 46, 35, 32 and 14 kDa, while membranes 4, 5, 6, 7 and 8 negative for Rose Bengal showed proteins of 67, 52, 46, 40, 36, 32, 14 kDa.

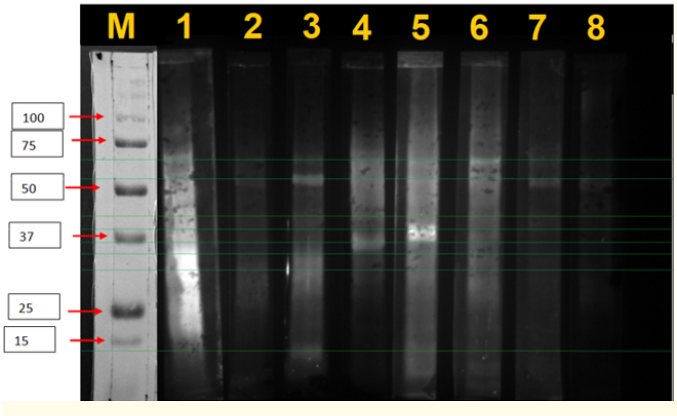

Figure 2: Representative Western blot of the proteins revealed by chemiluminescence of gel number 2. The 250 kDa molecular weight marker is indicated with M, serum samples 1-3 positive and 4-8 negative for Rose Bengal, respectively.

In gel number 3 (Figure 3), 9 samples were run, all of which were positive for Rose Bengal and showed proteins with molecular weights of 77, 75, 62, 50, 43, 37, 32, 29, 16 kDa.

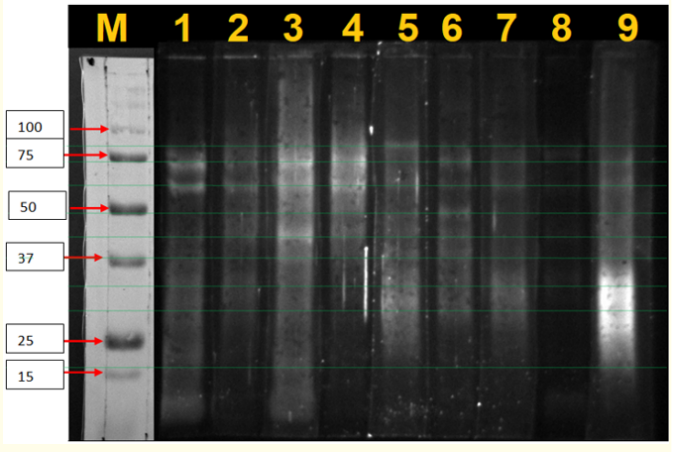

Figure 3: Representative Western blot of the proteins revealed by chemiluminescence of gel number 3. The 250 kDa molecular weight marker and serum samples 1-9 positive for Rose Bengal are indicated with M.

In gel number 4 (Figure 4), 9 samples were run, of which membranes 1, 2, 3 and 4 were positive for Rose Bengal, showing proteins of 76, 74, 62, 50, 43, 47, 36, 27, 24 and 13 kDa, on the other hand, membranes 5, 6, 7, 8 and 9 negative for Rose Bengal showed proteins of 76, 74, 62, 50, 43, 36, and 27 kDa.

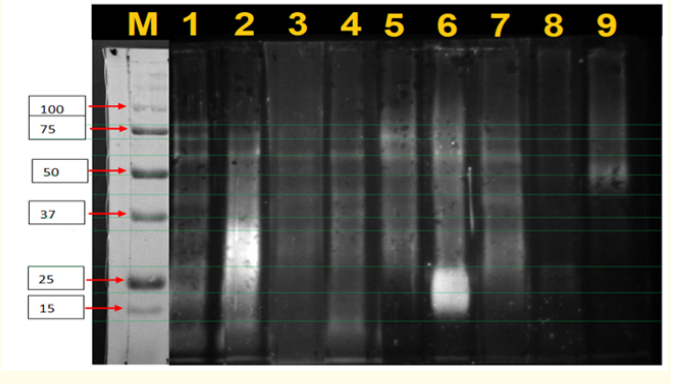

Figure 4: Representative Western blot of the proteins revealed by chemiluminescence of gel number 4. The molecular weight marker of 250 kDa is indicated with M, serum samples 1-4 positive and 5-9 negative for Rose Bengal, respectively.

Based on the molecular weights presented by the proteins visualized in the Western blot from the groups of samples positive and

negative for rose bengal, proteins were found with a wide diversity of molecular weights, both low and high. The most frequent proteins in samples positive for Rose Bengal were 75, 62, 52, 50, 43, 37 and 28 kDa respectively, while for samples negative for Rose Bengal they were 74, 62, 50, 43 and 36 kDa as represented in Table 2.

Protein molecular weight (kDa)	Presence of protein in samples positive for Rose Bengal	Percent	Presene of protein in samples positive for Rose Bengal	Percent
125	1/26	3.8%	0/10	0%
95	1/26	3.8%	0/10	0%
77	1/26	3.8%	0/10	0%
76	2/26	7.6%	1/10	10%
75	14/26	53.8%	0/10	0%
74	2/26	7.6%	4/10	40%
67	2/26	7.6%	1/10	10%
62	23/26	88.4%	3/10	30%
52	10/26	38.4%	2/10	20%
50	10/26	38.4%	3/10	30%
46	1/26	3.8%	1/26	10%
44	3/26	11.5%	0/10	0%
43	8/26	30.7%	3/10	30%
40	3/26	11.5%	0/10	0%
37	11/26	42.3%	0/10	0%
36	2/26	7.6%	4/10	40%
35	1/26	3.8%	0/10	0%
34	5/26	19.2%	0/10	0%
32	7/26	26.9%	2/10	20%
29	5/26	19.2%	0/10	0%
28	10/26	38.4%	0/10	0%
27	1/26	3.8%	2/10	20%
26	2/26	7.6%	0/10	0%
24	2/26	7.6%	0/10	0%
21	4/26	15.3%	0/10	0%
16	2/26	7.6%	0/10	0%
14	3/26	11.5%	1/10	10%
13	2/26	7.6%	0/10	0%

Table 2: Analysis of the presence of proteins revealed by WB in Rose Bengal positive and negative samples.

Discussion

The objective of this research was to evaluate the Western blot technique for the detection of lipopolysaccharides and glycoproteins from specific *Brucella* antigens in northeastern Mexico. Currently, there are specific diagnostic methods for Brucellosis in humans, serological, molecular or microbiological, the Bengal rose is still used as a diagnostic test for brucellosis [17,22], it is commonly used for primary screening because of its high sensitivity, low cost and simplicity [9], molecular tests allow us to know the species of the *Brucella* genus. In this regard, it was recently published that the results of multiplex PCR amplification of samples positive and negative for rose bengal showed bands that gave rise to the iden-

tification of genus and species of *Brucella*, the multiplex PCR was performed directly from clinical samples giving certainty. in the diagnosis, with which a sensitivity and specificity of 100% was obtained, so the PPV and NPV were 100% [23].

The expression of the lipopolysaccharides that constitute the O antigen and the endotoxin of *Brucella*, located in the external membrane of the bacterial cell envelope and play a very important role in the pathogenesis of the bacterial infection, as well as in the interaction with the host and their defense system. Basically, LPS is composed of a lipid portion highly conserved between species, called lipid A, immersed in the external face of the outer membrane of the bacteria, and a hydrophilic portion composed of sugars that presents great structural variability. Lipid A is responsible for the pathophysiological properties of endotoxins. The O polysaccharide, which is the outermost portion, gives the bacteria its serological specificity, which is evidenced by the serological reaction observed with the Rose Bengal antigen. In this regard, has been mentioned basic aspects about the proteins of the outer membrane of *Brucella* that are closely associated with lipopolysaccharides, and are called major proteins.

With regard to the Western-blot technique, as already mentioned, despite the disadvantages offered by serological methods such as rose bengal for the diagnosis of brucellosis, these have not been able to be replaced due to their demonstrable advantages, however, for some time we have been working on the search for an antigenic component that is unique and typical of the *Brucella* genus and that on paper leads to the generation of a test that is equally easy to execute as those described until today.

The Western-blot technique allows us, first, to extract the total proteins from the control strains, analyze their similarities in terms of protein components, subsequently, identify the pattern of immunogenic proteins using the sera from the samples of group I and II and finally evaluate if there are differences between the patterns of immunogenic proteins between the two study strains.

There are other proteins in smaller quantities called minor proteins. Major proteins are classified into three types according to their molecular weight: group 1 (80-94 kDa), group 2 (36-48 kDa) and group 3 (15-27 and 31-34 kDa) and are exposed in the outer membrane, but they are less accessible in the smooth strains than in the rough ones, due to the steric hindrance caused by the O chains of the LPS of the former, when the control strains used in In our study of rugose strains, the proteins belonging to the three groups were easily recognizable.

Based on the proteins visualized in the Western Blot, the proteins with the highest percentage of appearance in Rose Bengal-negative samples were 74, 62, 50, 43 and 36 kDa, of which the proteins of 62, 50 and 43 have a high percentage of presence in both positive and negative samples for Rose Bengal; On the other hand, the 74 and 36 kDa proteins have a low percentage of appearance in positive samples and a high percentage of appearance in negative samples, which indicates that these proteins are nonspecific and of little use as markers indicative of brucellosis.

The group of proteins with molecular weights of 75, 52, 37 and 28 kDa were those with the highest percentages of appearance in samples positive for Rose Bengal, observed in the Western Blot, the 52 kDa protein showed a percentage of presence of 38.4%. based on the 26 samples positive for Rose Bengal, but a negative Rose Bengal was also observed in 2 of the 10 representative samples, so it was discarded. In the case of proteins 75, 37 and 28, their presence was not observed in any of the Rose Bengal-negative sera.

Wareth and collaborators in 2016 found proteins specific for *B. abortus* detected in a range of 70-100 kDa, but there were no signals of proteins specific to *B. melitensis* in this range. On the other hand, they detected *B. melitensis*-specific proteins of 37, 27, 31, 30, 25, 25, 23 kDa in positive goat sera.

In another study Wareth and collaborators in 2016 point out 3 proteins as specific to *B. melitensis* in both goats and sheep, with molecular weights of 33 and 31 (two proteins) kDa respectively [24].

The 75 kDa protein that we detected in the Western blot was detected in 14 of the 26 sera positive for Rose Bengal, being the protein with the highest presence with a percentage of 53.8%, but as Wareth., *et al*. mentioned in 2016, no protein of High molecular weight can be established as a specific marker for *B. melitensis*.

On the other hand, the 37 and 28 kDa proteins are of low molecular weight, with the 37 kDa protein also detected in the study by Wareth and collaborators in 2016, the 28 kDa protein that we found was present in 10 of the 26 sera positive to Rose Bengal with

a percentage of appearance of 38,4% but it should be noted that no reference is made to this protein in the aforementioned studies. It is also documented that group 3 proteins are antigenic components similar to those found in other Gram (-) bacteria with which *Brucella* has a cross reaction and this is perhaps why they tested positive in the rose bengal technique. It is pertinent to note that the species of *B. melitensis*, *B. abortus* and *B. suis*, are transmitted between animals both vertically and horizontally, causing abortion and infertility in their natural primary host, goats, cattle and pigs, respectively. Despite the effort and money invested in surveillance programs against brucellosis, seronegative animals hinder the control program and could facilitate contagion to the environment and spread the disease to susceptible host [12].

The practice of transhumance (the seasonal movement of people with their livestock from one pasture to another to improve grazing) among herders, uncontrolled cross-border movement of animals and mixing of livestock during veterinary interventions are common and can facilitate the spread of the illness [25]. Acute and chronic symptoms of the disease can lead to significant loss of work days and subsequent disparity in the socioeconomic status of infected individuals and their families [3]. The main precaution that can be taken for the prevention of brucellosis infection is the elimination of raw meat and unpasteurized animal products, including milk and cheese, and the promotion of personal protection, such as using thick gloves, spectacles, and dresses for individuals who are in direct contact with animals [17]. The key element in the brucellosis elimination measures is the timely isolation of infected animals [26]. According to Awais and collaborators (2024), an effective disease surveillance mechanism should be devised to determine the load of disease in livestock and human populations at regional and national levels for its efficient control. In general, a highly effective vaccine has not been developed. In the absence of an effective vaccine, it is difficult to determine the seroprevalence of brucellosis in cattle. Availability of an effective vaccine will contribute towards a sustainable strategy for control of this zoonotic disease [13]. Over the past few decades, *Brucella* cell wall proteins screened with S-LPS have become the focus of study as promising immunogens for vaccine development and as components for creating specific diagnostic antigens [26]. Regarding this Pinn-Woodcock and collaborators (2023) recommend that due to the zoonotic nature of brucellosis, its management requires the awareness of medical professionals in both the human health and veterinary sectors, including knowledge of the exposure risks, diagnostic limitations, treatment practices, and preventative surveillance programs in place [2]. The risk factor that prevails for human beings is the consumption of unpasteurized dairy products, so it is necessary to provide technical advice to small producers on the knowledge of brucellosis, how to prevent the infection in order to not only control it but eradicate it from brucellosis endemic areas.

For future research, it is advisable to use multiplex PCR for diagnosis of the different species of the *Brucella* genus because it simultaneously amplifies different target sequences, as well as the simultaneous identification of various genes. It is recommended to process the multiplex PCR with techniques such as Western-blot that detects specific proteins because there are cases in which genes that encode toxins or other virulence factors are detected.

Currently, brucellosis is a public health problem in the Comarca Lagunera region of Coahuila and Durango, affecting the entire population that maintains direct contact with animals infected by *Brucella* spp, or indirectly in the consumption of their unpasteurized products. It is also a problem economically because sick animals have to be sacrificed and are losses for the owners of the stables.

There is no specific diagnostic method for brucellosis in both people and animals; serology is used as a tool to locate the presence of antibodies against *Brucella* spp but does not discriminate between individuals who currently have the infection or memory antibodies. by vaccination, therefore it is impossible to know the stage in which the disease is, on the other hand, there is the problem in which cross reactions occur with other pathogenic agents antigenically similar to *Brucella* spp. Therefore, it is important to explore different techniques to guarantee a more accurate diagnosis of this disease.

Conclusion

In the present work, the exposure of *B. melitensis* lipopolysaccharide was revealed, evidenced by the serological reaction observed with the Rose Bengal antigen and by the Western blot technique, 28 immunodominant proteins were detected in sera previously evaluated with the Rose Bengal test, observed that of the group of 4 goat breeds studied (Nubia, Toggenburg, Saneen and Alpina) the exclusive presence of any protein was not found in a specific breed, assuming that *Brucella melitensis* does not discriminate between breeds.

It is proposed that the 37 kDa protein is the most suitable to be used as a good marker of *B. melitensis* in goats and can be taken

as a reference for subsequent studies in order to establish specific biomarkers for the diagnosis of this etiological agent.

Conflict of Interests

The authors have not conflict of interest.

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