Vol. 22, No. 1, Spring, 2026, 68-82
DOI: 10.22055/ivj.2025.531580.2809
Journal Homepage: http://www.ivj.ir/

Original Article

Evaluation of the cross-reactive antibodies among *Brucella* strains: validation of serological detection tests of Brucellosis

Farangis Nargesi[1], Mohammad Khosravi[2*], Darioush Gharibi[3], Mahdi Pourmahdi Borujeni[4] and Masoud Ghorbanpoor[5]

[1] PhD Student in Bacteriology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Associate Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[4] Professor, Department of Food hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[5] Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Received: 28.06.2025 Accepted: 06.10.2025

Abstract

The present study aimed to investigate the antibody cross-reactions in sheep serum against vaccine strains and pathogenic strains of *Brucella*, as well as validation of the agglutination results with ELISA using both vaccine and wild strains of *Brucella*. The used vaccine and wild strains were *RB51*, *Rev1*, *B. melitensis*, and *B. abortus*, all of which were confirmed by PCR test. A total of 81 sheep serum samples were included in the study, encompassing both positive and negative reactions in the agglutination tests. Following the characterization of the sera, indirect ELISA tests utilizing the lipopolysaccharide of the bacteria were employed to validate the agglutination results and to statistically assess the serum cross-reactions against the vaccine and wild strains of *Brucella.* Out of 81 evaluated suspected serum samples, the Rose-Bengal test yielded 61 positive and 20 negative results. The Wright and Wright-2ME tests revealed 38 and 36 positive, and 43 and 45 negative results, respectively. According to the ROC curve analysis, the highest area under the curve was 84% for IRIBA, 75% for Rev1, 70% for *B. melitensis*, and 68% for *B. abortus*. Statistical analysis indicated that the strain of antigen in ELISA tests had a significant effect on S/P values. The mean and standard error of the S/P values were as Rev1 vaccine 0.54 ± 0.03, *B. melitensis* 0.82 ± 0.02, IRIBA vaccine 0.82 ± 0.04, and *B. abortus* 0.98 ± 0.04. The highest sensitivity and specificity were achieved using *B. melitensis* and IRIBA antigens, respectively. The indirect ELISA utilizing both vaccine and wild strains of *Brucella* demonstrated appropriate sensitivity and specificity, suggesting its cross-reactions and potential as a reliable diagnostic method for vaccinated and infected sheep.

Key words: Brucella, Sheep, Vaccine, Cross-reaction, ELISA

Introduction

Brucellosis is a bacterial disease caused by various species of *Brucella*, primarily affecting cattle, pigs, goats, sheep, and dogs, but it can also infect humans (Priyantha, 2021). *Brucella* is most concentrated in the uterus of pregnant animals. Aborted fetuses, placental membranes, and uterine secretions serve as the primary sources of infection (Jiao et al, 2021). Animals can become infected by consuming contaminated food and water or through contact with aborted fetuses and uterine secretions (Kiros et al, 2016). Common sources of infection in humans include contact with aborted animal products, consumption of unpasteurized dairy products, ingestion of raw or

* **Corresponding Author**: Mohammad Khosravi, Associate Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: m.khosravi@scu.ac.ir

© 2020 by the authors. Licensee SCU, Ahvaz, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0 license) (http://creativecommons.org/licenses/by-nc/4.0/).

undercooked meat, exposure to laboratory cultures and tissue samples, and accidental injection of brucellosis vaccines (Pal et al, 2017). Consequently, brucellosis is a zoonotic disease that poses significant public health risks and economic challenges in countries where it is endemic. The wide range of animals that can act as vectors, along with the numerous pathways contributing to its spread, complicates prevention efforts (Berhanu et al, 2020). Brucellosis is classified as a notifiable disease, meaning that any occurrence in a country, region, or sector must be reported, even in the absence of clinical signs; this reporting requirement is established by the World Organization for Animal Health (Perez et al, 2024).

The number of infected animals could be elevated due to the country's extensive borders, lack of monitoring of livestock imports, large population of nomads, traditional animal husbandry practices, inadequate supervision of dairy production and distribution, and absence of regular vaccination and monitoring of disease eradication programs such as testing and slaughtering animals. Studies conducted in various parts of Iran have reported the prevalence of the disease ranging from 2.3% to 9.53% (Sadeghifard et al, 2023).

B. melitensis is the most common *Brucella* species shared between humans and animals, followed by *B. abortus*, *B. suis*, and *B. canis*. Recent studies suggest that *B. neotomae* and *B. inopinata* may also have the potential for human-animal transmission, indicating a potentially wider distribution of human-animal potential within the genus *Brucella* (Sadaqat et ai., 2024). Genomic analysis has revealed a highly conserved genomic structure among different *Brucella* species. The genomic similarity within the genus *Brucella* is above 97% (Yang et al, 2024). The pathogenicity of *Brucella* mainly results from its ability to evade the host defense system and survive for long periods in specialized phagocytes such as macrophages and dendritic cells (DCs), as well as placental trophoblast cells (Copin et al, 2015; Martirosyan et al, 2013). The response to *Brucella* species involves the full range of immune system responses, including innate to acquired immunity (Golding et al, 2001).

Brucella melitensis, the etiological agent of brucellosis in sheep, has been recognized as the principal pathogen responsible for the global dissemination of human brucellosis, owing to its infective dose being approximately 10,000 times lower than that of *Brucella abortus* (Yaeger and Holler, 2007; Whatmore, 2009). In contrast to cow’s milk, sheep’s milk is frequently used in the production of traditional unpasteurized dairy products, thereby increasing the risk of brucellosis transmission to consumers and contributing to the occurrence of milk-borne Malta fever (Pandya et al, 2007). In developing countries, traditional and cultural practices have led to the widespread acceptance of raw milk and unpasteurized dairy products derived from various animals—including cows, goats, sheep, donkeys, buffaloes, yaks, and camels—due to their greater affordability and accessibility (Falenski et al, 2011). Similarly, in Iran, the consumption of unpasteurized dairy products represents a major risk factor for human brucellosis infection (Sofian et al, 2008).

The laboratory tests are necessary for diagnosis of brucellosis, because it is difficult to correctly diagnose the disease based only on non-specific and sometimes atypical clinical symptoms (Keyvanfar et al, 2021). The method of diagnostic testing depends on the aim of the test, as diagnosis for disease confirmation, national screening programs, certification for international trade, and surveillance of the disease in countries where it has been eradicated. Diagnostic tests of brucellosis are divided into two categories (Smirnova et al, 2013). The direct method includes tests to detect the causative agent, such as various staining

methods, culture methods, and polymerase chain reaction (PCR). The indirect category includes tests used to detect the immune response, such as the Rose Bengal test (RBT), complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), serum agglutination test (SAT), and milk ring tests (Poester et al, 2010). Indirect tests may be performed in laboratory conditions, such as blood and milk tests, or in vivo, such as skin tests. Treatment of animal's brucellosis is not recommended because it is ineffective, costly, and carries the risk of developing antibiotic-resistant strains of *Brucella*. In addition, treated animals may continue to be carriers of the bacteria. Therefore, the prevention and control of brucellosis is based on a combination of methods such as vaccination, identification, testing, elimination, quarantine, biosecurity, and public education (Rehman et al, 2025). Vaccination is one of the most important tools to reduce the prevalence and incidence of brucellosis in endemic areas. The most commonly used vaccines are live attenuated strains of *B. abortus* and *B. melitensis* such as strains S19, RB51 and REV1. These vaccines induce immunity in cattle by stimulating humoral and cellular responses. However, these vaccines also have disadvantages, such as causing abortion in pregnant animals, inducing antibodies that interfere with serological diagnosis, and potentially could infect humans (Dorneles et al, 2015). Removal of infected animals helps to prevent further transmission and contamination (Daro et al, 2024).

Lipopolysaccharide is recognized as a key virulence factor that plays a crucial role in bacterial pathogenesis, biological phenotypes, and immune evasion (Stranahan et al, 2021). Based on the integrity of the O antigen structure in the LPS, *Brucella* is classified into two groups: Soft and rough. Rough strains are further subdivided into two categories: fully rough and semi-rough. In fully rough Brucella, the entire O antigen structure in the LPS is absent, whereas in semi-rough Brucella, part of this structure remains. Notably, LPS not only contributes to the residual virulence of commercial *Brucella* vaccines but also complicates the serological differentiation between infected and vaccinated animals (DIVA) (Abdelgawad et al, 2023; Lalsiamthara et al, 2015; Li et al, 2015). Live attenuated vaccines, such as *B. abortus* S19, *B. melitensis* Rev.1, and *B. abortus* RB51, remain the cornerstone of brucellosis control programs (Heidary et al, 2022). However, their use presents significant challenges. Vaccines like S19 and Rev.1 pose potential risks to human health due to their residual virulence. Additionally, their smooth LPS phenotype induces a strong antibody response, which hinders DIVA detection. In contrast, the RB51 rough strain, which lacks the O antigen, has been introduced as a DIVA-compatible vaccine. However, its resistance to rifampicin limits treatment options in cases of accidental human exposure (Heidary et al., 2022). Furthermore, RB51, derived from the *B. abortus* strain, is primarily used for cattle immunization. Conversely, *B. melitensis* primarily affects goats and sheep, and no current DIVA vaccine is available to manage *B. melitensis* in small ruminants—highlighting the urgent need for new vaccine development (Haine et al, 2005). The present study aimed to investigate the antibody cross-reactivity between vaccine strains and pathogenic strains of *Brucella*.

Materials and methods

Preparation of Bacterial strains

The *Brucella* bacteria used in this study were vaccine strains of *Brucella abortus* and *Brucella melitensis*, as well as previously isolated bacteria from clinical samples of ruminants. The vaccine strains live *Brucella melitensis* strain REV1 and live *Brucella abortus* strain IRIBA were prepared from the Razi vaccine and serum research institute, Karaj, Iran. The wild strains of *Brucella abortus* and *Brucella melitensis* were provided from archive of microbiology

department of veterinary faculty of Tehran and Shahid Chamran University of Ahvaz, respectively. The bacterial strains were cultured on blood-enriched Brucella-agar medium. The cultured plates were incubated at 37ºC and in the presence of CO_2 in a candle jar for a minimum of 48 hours, and the plates were assessed for bacterial growth. The *Brucella* strains were confirmed by Gram staining, Oxidase, catalase, and routine biochemical tests on suspected colonies (Markey et al, 2013). Bacteria exhibiting initial characteristics of *Brucella* were subsequently identified to the species level using multiplex PCR, based on the method developed by Lopez-Goni et al, (2008). This PCR assay is capable of distinguishing all *Brucella* species, as well as differentiating vaccine strains from wild-type strains. Species- and strain-specific primers were employed to ensure accurate differentiation. All biovars of *Brucella abortus* lack the omp31 gene and exhibit a 25 kb deletion, while the S19 vaccine strain additionally harbors a 702 bp deletion in the ery operon. The RB51 vaccine strain is characterized by disruption of the wboA gene due to the insertion of the IS711 element and the absence of portions of the wbo operon, distinguishing it from natural strains. In the case of *B. melitensis*, the Rev1 vaccine strain carries a unique mutation in the rpsL gene, which is absent in reference strains. Other species-specific differences include a 976 bp deletion in chromosome I of *Brucella canis*, a 2.2 kb deletion in chromosome II of *Brucella neotomae*, and a unique 2.6 kb fragment in *B. suis* that is not present in other species. Marine *Brucella* isolates exhibit the presence of the IS711 element downstream of the bp26 gene. These genetic variations served as specific markers for primer design, enabling precise discrimination of *Brucella* species and strains (Lopez-Goni et al, 2008).

Performing a multiplex PCR reaction to confirm *Brucella* strains

The strains of *Bacillus abortus* and *Bacillus melitensis*, as well as two vaccine strains were identified using the multiplex PCR. The materials were mixed with the following values and order for the PCR reactions with the total volume of 50 µL; dH2O 4 µL, Master Mix (Mgcl$_2$ 2 mM, Ampliqon, Denmark) 25 µL, DNA sample 5 µL, forward primer 1µL, reverse primer 1µL of the evaluated *Brucella* species. The sequences of the used primers (Table 1), annealing temperature and time programs of the PCR reactions were performed according to Lopez-Goni et al, (2008).

Table 1: Sequences of the used primers for identification of the *Brucella* species

Primer	Sequence (5´- 3´)	Amplicon size (bp)
BMEI0998F	ATC CTA TTG CCC CGA TAA GG	1,682
BMEI0997R	GCT TCG CAT TTT CAC TGT AGC	
BMEI0535F	GCG CAT TCT TCG GTT ATG AA	450 (1,320b)
BMEI0536R	CGC AGG CGA AAA CAG CTA TAA	
BMEII0843F	TTT ACA CAG GCA ATC CAG CA	1,071
BMEII0844R	GCG TCC AGT TGT TGT TGA TG	
BMEI1436F	ACG CAG ACG ACC TTC GGT AT	794
BMEI1435R	TTT ATC CAT CGC CCT GTC AC	
BMEII0428F	GCC GCT ATT ATG TGG ACT GG	587
BMEII0428R	AAT GAC TTC ACG GTC GTT CG	
BR0953F	GGA ACA CTA CGC CAC CTT GT	272
BR0953R	GAT GGA GCA AAC GCT GAA G	
BMEI0752F	CAG GCA AAC CCT CAG AAG C	218
BMEI0752R	GAT GTG GTA ACG CAC ACC AA	
BMEII0987F	CGC AGA CAG TGA CCA TCA AA	152
BMEII0987R	GTA TTC AGC CCC CGT TAC CT	

Preparation of rabbit's anti-sheep IgG

Extraction of IgG antibodies from three ovine serum samples was conducted using ion exchange chromatography following an optimized method; the concentration and purity of the isolated antibody was assessed using Bradford analysis and Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), respectively (Khosravi et al, 2021). The purified IgG mixed with adjuvant were injected to two rabbits to generate hyperimmune serum. In the initial injection phase, 0.5 mL of the sheep IgG (500 µg/mL) along with 0.5 mL of montanide ISA720 adjuvant (Sigma, M8819) were injected into the leg muscles of the animals. Three booster injections of 300 µg/mL, in the same volume as the initial injection, were administered at 14-day intervals. Immunization was confirmed using an in-house ELISA test as described in follow section. Following the immunization, anesthesia was induced by injecting a solution of ketamine-xylazine at a concentration of 25:5 mg/mL, at a dosage of 1 mL per kilogram of body weight. After that the blood samples were taken from anesthetized animals and the hyperimmune serum against sheep IgG was separated. The total IgG was extracted from hyperimmune serum using ion exchange chromatography as previously (Yousefinejad et al, 2024). The concentration, purity and reactivity of the extracted rabbit anti-ovine IgG was evaluated using Bradford, SDS-PAGE and ELISA, respectively. Briefly, electrophoresis was performed with a vertical electrophoresis tank utilizing the Laemmli discontinuous SDS–PAGE on 11 % of the separating and 4 % of the stacking polyacrylamide gel. The polyacrylamide gels were stained for 30 minutes with Coomassie blue staining solution followed by de-staining with 10 % acetic acid, 40 % methanol solution overnight.

Evaluated serum Samples

The serum samples were collected from the 81 sheep of Khuzestan, Chaharmahal-Bakhtiari, and Gilan province with positive results in the Rose Bengal test in at least 40 animals. Serological tests for detection of antibodies in sheep serum were Rose Bengal, Wright, 2-Mercaptoethanol-Wright (2ME-Wright) and ELISA tests. The serological tests were performed according to OIE terrestrial manual (OIE, 2021). To perform the Rose Bengal test, a drop of serum equivalent to 30 µL and a drop of antigen were mixed on a 1.5 cm diameter in a perforated slide, and the results were documented within a maximum of 4 minutes. Positive samples were graded from +1 to +4 according to the intensity and time of positive reaction. The Wright and 2ME-Wright tests were performed on serum samples by serial dilution with a dilution factor of 1/2 and an initial dilution of 1:10, in sterile test tubes in accordance with the instructions of the Razi Vaccine and Serum Research Institute, Iran (Khosravi et al, 2018).

Extraction of *Brucella* lipopolysaccharides

LPS extraction was performed according to Davis et al, (2012); in brief the pelleted bacteria (1.5 mL of OD600=0.5) were dissolved in 200 µL of 1x SDS buffer and pipetted. The suspended bacteria were boiled in a water bath for 15 minutes. The solution was then cooled at room temperature for 15 minutes. The Proteinase K 10 µL was added to each sample; then, the samples were incubated for 3 hours at 59°C. The ice-cold Tris-saturated phenol was added 200 µL to each sample. Each sample was mixed using vortex for approximately 5 to 10 seconds. The samples were incubated for 15 minutes at 65°C and vortexed. After cooling to room temperature, 1 mL of diethyl ether was added to each sample and mixed for 5 to 10 seconds using a vortex mixer. The samples were centrifuged at 2600 × g for 10 minutes, and the pale blue precipitates were collected as LPS. The carbohydrate concentration was determined using the phenol–sulfuric acid method, with glucose used to prepare the standard samples.

Detection of antibody titer against *Brucella* strains using in-house ELISA

The indirect ELISA tests were performed using LPS antigen of vaccine or pathogenic *Brucella abortus* and *Brucella melitensis* strains. The used polystyrene ELISA plates (JetBiofil, China) were activated initially by treatment with 100 µL of 1% glutaraldehyde solution for 1 hour. After three washing steps of the wells using PBS-Tween 20 (0.05%) (PBS-T), based on initial checkerboard tests, all LPS samples were diluted to a concentration of 10 µg/mL using carbonate-bicarbonate buffer at pH 9.6, 100 µL of each sample was added to each well of the plate. The plates were incubated at 4°C overnight; afterward, the plate was washed three times with PBS-T, and the wells were blocked by adding 250 µL of 4% skim milk to each well. The plates were then incubated at 37°C for 2 hours. The plate was washed three times as before. Serum samples, diluted 1:50 with PBS buffer according to the checkerboard titration results, were added to each well, and the plate was incubated at room temperature for one hour. Following another wash, 100 µL of the rabbit anti-sheep IgG (100 µg/mL) was added. After incubation for one hour and another washing step, HRP-conjugated antibody against rabbit IgG (1/20.000) (Immuno Chemistry Technologies company, USA, HRP AffiPure Goat anti-Rabbit IgG Fc, Catalog Number: 6293), was added to each well and incubated for one hour before another washing step. Next, 50 µL of tetramethylbenzidine (TMB) substrate was added to each well. After incubating the plate for 10 minutes at room temperature, the reaction was stopped by adding 50 µL of stop solution. The optical density values were measured spectrophotometrically at a wavelength of 450 nm.

Statistical Calculation

The obtained data were analyzed with SPSS software (Version 22.0. Armonk, NY: IBM Corp) descriptively and analytically. Comparison of diagnostic methods was performed with Cochran and McNemar tests, kappa statistic and ROC (Receiver operating characteristic) curve analysis. Comparison of S/P values on ELISA test was performed using ANOVA and Tukey test. The $P \leq 0.05$ differences were considered statistically significant.

Results

The *Brucella* strains were successfully cultured and confirmed; as shown in Figure 1A and Figure 1B, the morphologic appearance and molecular pattern of the bands was produced in PCR reactions. The carbohydrate detection assay revealed that 4–6 mg of lipopolysaccharide was obtained per extraction, indicating a consistent LPS content across all samples.

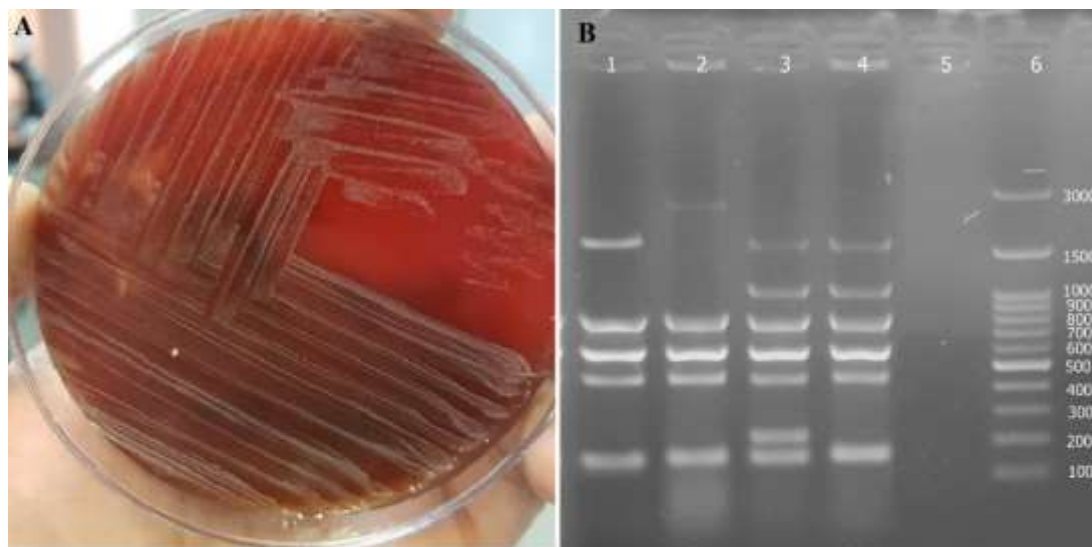

Figure 1: The confirmation of *Brucella* strains. A) The morphologic appearance of the cultured *Brucella* strain. B) Confirmation of the *Brucella* strains using multiplex PCR. Confirmation of the *Brucella* strains using multiplex PCR. Lane 1: *Brucella abortus*, Lane 2: RB51, Lane 3: Rev1, Lane 4: *Brucella melitensis*, Lane 5: negative control and Lane 6: molecular leader 100bp+3kb. Using the primer pairs, *Brucella melitensis* produced fragments of 1682, 1071, 794, 587, 450, and 152 bp, whereas the Rev1 vaccine strain additionally generated a 218 bp fragment, allowing it to be distinguished from the virulent strain. The *B. abortus* and RB51 vaccine strains were identified by the absence of the 1682 bp fragment; additionally, RB51 has an extra band with a molecular weight of 2524 bp.

Production of polyclonal antibody against sheep IgG

The purification process of IgG from sheep sera was successfully accomplished. In this procedure, approximately 8 mg of purified IgG was obtained per milliliter of serum. The purification outcome of antibodies derived from hyperimmunized rabbit serum is demonstrated in Figure 2A, as assessed by SDS-PAGE. The immunization of rabbits against sheep IgG was successfully confirmed using ELISA (Figure 2B).

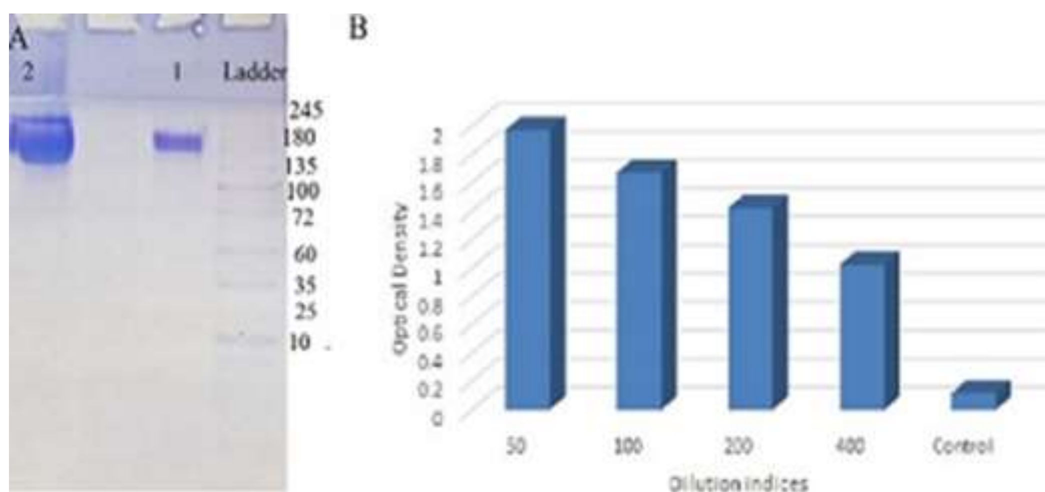

Figure 2: Production of rabbit's IgG against sheep IgG. A) Purification of IgG antibody from hyperimmunized rabbits against sheep IgG. Lane 1. The purified sheep IgG, Lane 2. The purified rabbit's anti-sheep IgG. B) The antibody titer of the hyperimmunized rabbits against sheep IgG on ELISA.

Detection of anti-*Brucella* titer

Out of evaluated 81 suspected serum samples, Rose-Bengal test resulted in 61 positive and 20 negatives; Wright and Wright-2ME tests resulted in respectively, 38, 36 positive and 43, 45 negatives (Tables 2 and 3 and Supplementary Table 1). The results of the McNemar test indicated a significant difference between Rose Bengal and Wright tests. The kappa statistic for these two methods was calculated to be 0.45. Based on this comparison, 38 cases tested positive using both Rose Bengal and Wright tests, 20 cases tested negative with both methods, and 23 cases tested positive only with Rose Bengal test. The Spearman correlation coefficient between Rose Bengal and Wright titers was 0.8, indicating a strong, direct, and significant correlation (P≤0.001). The positive and negative samples in Wright and Rose Bengal tests were respectively, 38 and 43 samples, considered as real positive and negative samples.

Table 2: **Comparison of Wright and Rose Bengal tests in sheep serum samples for diagnosis of brucellosis**

Rose Bengal \ Wright	Positive	Negative	Total
Positive	38	23	61
Negative	0	20	20
Total	38	43	81

There was a significant difference between Rose Bengal and 2ME-Wright and the agreement beyond chance was 0.42. Based on this comparison, 36 cases were positive in both Rose Bengal and 2ME-Wright, 20 cases were negative in both methods, but 25 cases were only positive for Rose Bengal. Spearman's correlation coefficient between Rose Bengal and 2ME-Wright titers was 0.59 (a moderate, direct, and significant correlation) (P≤0.001).

Table 3: Comparison of 2ME-Wright and Rose Bengal tests in sheep serum samples for diagnosis of brucellosis

Rose Bengal \ Wright 2me	Positive	Negative	Total
Positive	36	25	61
Negative	0	20	20
Total	36	45	81

Detection of *Brucella* strain's cross reaction

A total of 43 serum samples were negative in both Rose Bengal and Wright agglutination tests; out of these samples, 21 samples were negative in ELISA tests using four strains of *Brucella*. Only, one positive sample in both agglutination tests was negative in ELISA tests with the four strains. Out of 22 negative serum samples in the four ELISA tests, 6, 2, and 3 samples yielded positive results using the Rose-Bengal, Wright, and 2ME-Wright tests, respectively. Additionally, 11 cases were positive in all four ELISA tests. The *B. abortus* and IRIBA antigens produced consistent results, showing 45 negative and 13 positive outcomes. The *B. abortus* and REV1 antigens also yielded similar results, with 47 negative and 13 positive outcomes. Lastly, the *B. abortus* and *B. melitensis* antigens showed consistent results, including 25 negative and 20 positive outcomes. Also, the 21 samples that were positive in the ELISA test using *B. melitensis* antigens, returned negative results for other strains.

B. melitensis and IRIBA antigens yielded similar results, including 24 negative and 28 positive outcomes. The Rev1 and IRIBA antigens also produced comparable results, with 48 negative and 23 positive outcomes. Additionally, the 2 samples that were negative in the ELISA test using Rev1 antigens showed positive results for other strains. Furthermore, 2 samples that were positive in the ELISA test using Rev1 antigens returned negative results for other strains. The 2 samples that were positive in the ELISA test using Rev1, *B. melitensis*, and *B. abortus* antigens showed negative results for the IRIBA strain, while one

sample that was positive in the ELISA test using IRIBA antigens returned negative results for other strains. The 5 samples that were positive in the ELISA test using *B. melitensis* and *B. abortus* antigens showed negative results for both the IRIBA and Rev1 strains. The 12 samples that were positive in the ELISA test using Rev1, *B. melitensis*, and IRIBA antigens returned negative results for the *B. abortus* strain. Lastly, the 3 samples that were positive in the ELISA test using *B. melitensis* and IRIBA antigens showed negative results for both the *B. abortus* and Rev1 strains.

The highest area under the ROC curve was found to be 0.84 for IRIBA followed by 0.75 for Rev1, 0.70 for *B. melitensis*, and 0.68 for *B. abortus*. As a result, detecting positive and negative cases using IRIBA antigens in ELISA test, resulted in more agreement with agglutination tests. The cut-off value for ELISA test by using different antigen of *Brucella* strains was also determined to establish the maximum sensitivity and specificity (Figure 3 and Supplementary Table 2).

The ELISA using Rev1 antigens, identified 23 out of 38 positive and 39 out of 43 negative results accurately. Therefore, there is a discrepancy between Rev1 and reality ($p \leq 0.05$), with a kappa value of 0.52. The ELISA using *B. melitensis* antigens, detected 35 positive samples out of total 38 positive samples and 22 negative samples, out of total 43 negative samples; the Kappa statistic was equal to 0.42. The ELISA using IRIBA antigens, detected 26 positive samples out of total 38 positive samples and 40 negative samples, out of total 43 negative samples; the Kappa statistic was equal to 0.62. The ELISA using *B. abortus* antigens, detected 16 positive samples out of total 38 positive samples and 39 negative samples, out of total 43 negative samples; the Kappa statistic was equal to 0.34 (Table 4 and Supplementary Table 3).

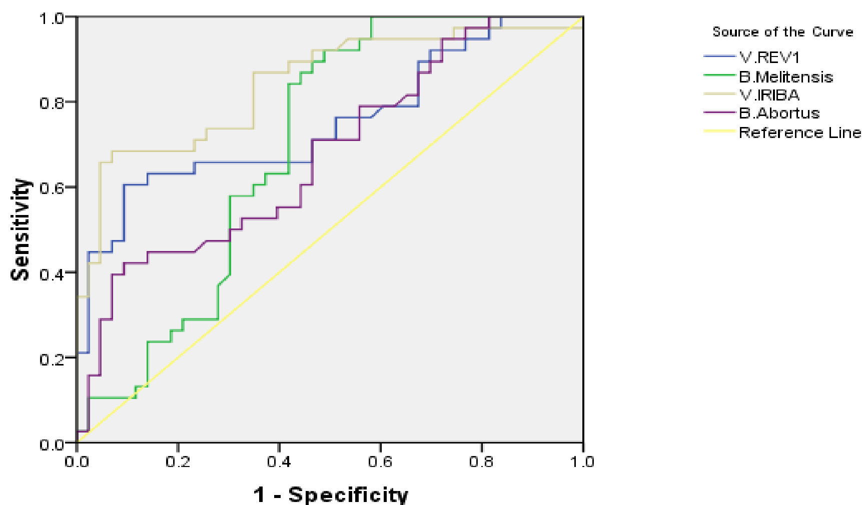

Figure 3: Statistical ROC analysis to determine the sensitivity and specificity of ELISA for diagnosis of brucellosis using membrane antigens from pathogenic and vaccine strains of *Brucella* for detection of positive and negative sheep serum samples.

Table 4: Compression of ELISA test on sheep serum samples for detection of brucellosis using lipopolysaccharide of vaccines and virulent strains of *Brucella*. Se: sensitivity; Sp: specificity, PPV: positive predictive value, NPV: negative predictive value

	Se	Sp	PPV	NPV	Agreement
B. Abortus	42.10	90.7	85.18	72.22	67.90
B. Melitensis	92.11	51.16	62.5	88	70.37
Rev1	60.5	90.7	85.18	72.22	76.54
IRIBA	68.42	93.02	89.65	76.92	81.48

Comparison of S/P values

According to the statistical analysis, the strain antigen in ELISA tests (P≤0.001) had a significant effect on S/P values. The mean and standard error of the S/P values were as REV1 vaccine 0.54 ± 0.03, *B. melitensis* 0.82 ± 0.02, IRIBA vaccine 0.82 ± 0.04, and *B. abortus* 0.98 ± 0.04 (Supplementary Tables 4 and 5). The S/P value by using the REV1 antigens in ELISA test was significantly lower than the other S/P values (P≤0.001), as well as lower for *B. melitensis* compared to *B. abortus* (P≤0.001). Additionally, S/P value was significantly lower, for the IRIBA vaccine, than *B. abortus* (P≤0.001). However, there was no significant difference in the S/P value between *B. melitensis* and the IRIBA vaccine (P>0.05) (Figure 4A).

Comparison of S/P values regardless of serum positivity or negativity showed a significant difference between the antigens (P≤0.001). REV1 had significantly lower S/P values compared to the others (P≤0.001). Additionally, *B. abortus* had significantly higher S/P values than *B. melitensis* and IRIBA (P≤0.001), but there was no significant difference between S/P values of *B. melitensis* and IRIBA (P>0.05) (Figure 4b).

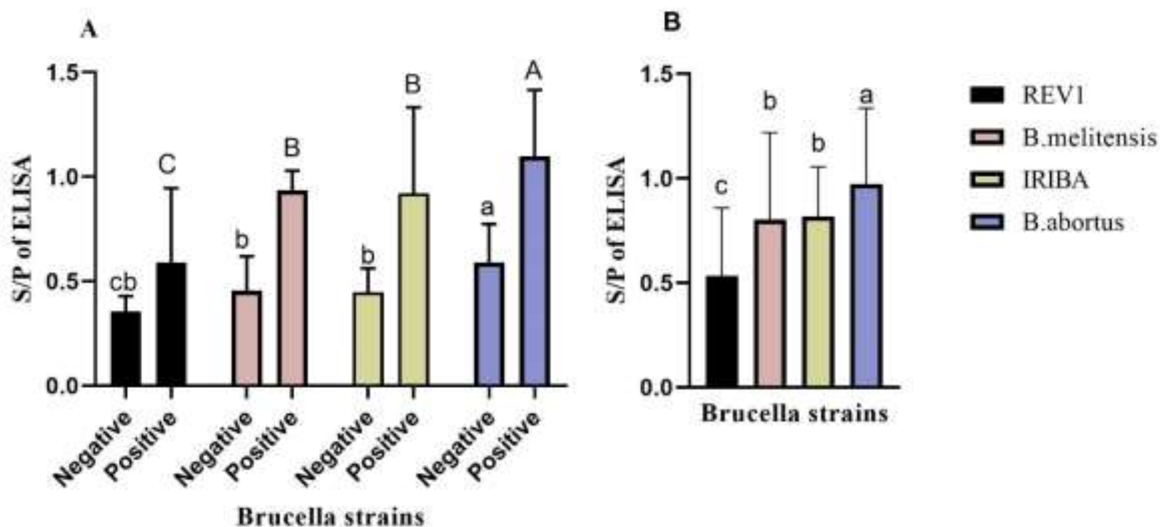

Figure 4: Comparison of S/P values of ELISA on sheep serum for detection of brucellosis using membrane antigens of vaccine and virulent strains of *Brucella.* A) Comparison of S/P values in accordance to of positive or negative results. B) Comparison of S/P values regardless of positive or negative results. A statistically significant difference was shown compared to the other strains with different letters (P>0.05).

Discussion

Effective diagnosis and vaccination remain essential issues for controlling of the brucellosis. Among the available vaccines, *B. melitensis* strain Rev1 is commonly used for sheep vaccination; however, it has known drawbacks, including the risk of abortion in pregnant animals and interference with serological diagnostics. The IRIBA vaccine has been developed for cattle vaccination as an alternative to reduce such limitations while maintaining protective efficacy. In the current study, the main serological methods were evaluated for detection of sheep brucellosis using wild and vaccine strains.

Serological tests showed varying performance; the Rose Bengal Test (RBT) resulted in higher positivity than Wright and 2ME Wright tests, although correlations between them were modest. Among ELISA tests, the highest specificity was obtained by using IRIBA antigens, suggesting better diagnostic of negative sheep infection to brucellosis. Kappa statistics further

supported the superior agreement of IRIBA results with the agglutination test results. Using the *B. melitensis* strain in ELISA offer an improved diagnostic sensitivity compared to other antigens. Its lower cross-reactivity and higher agreement in positive cases with agglutination results make it a promising strain for diagnosis of sheep brucellosis using ELISA. According to the results, out of a total of 56 positive samples were using *B. melitensis* in ELISA, 21 samples exhibited positive results exclusively with this strain, indicating a difference in cross-reactivity among some of the positive samples. This finding supports the suggestion of variation in *B. melitensis* serotypes. Among the evaluated *Brucella* strains, Rev1 and IRIBA demonstrated higher cross-reactivity compared to the other strains. Additionally, two samples were positive exclusively with the Rev1 strain, and one sample was positive only with the IRIBA strain, suggesting a potential infection of these animals with these vaccine strains. Since the present study focused on four endemic *Brucella strains* that were not fully represented in the available commercial kits, which were not used for comparative evaluation.

Banyasz et al. (2025) developed a Western blot-based diagnostic test, to resolve false-positive serological reactions (FPSR) in brucellosis testing, which often lead to unnecessary culling and quarantine. Their method successfully distinguished true *Brucella*-positive sera from samples containing cross-reactive antibodies produced against other gram-negative bacteria, achieving 100% sensitivity and specificity with high reproducibility (CV% = 1.36). These findings are consistent with the current study, where serological tests such as Rose Bengal, Wright, and 2ME-Wright identified some cases as positive, yet ELISA did not confirm the positive results. It highlights the need for combining agglutination test and other confirmatory diagnostic methods to improve brucellosis diagnosis accuracy (Banyasz et al, 2025). The current study suggests that ELISA test using *Brucella* strains could detect the caused strain and reveal false positive or false negative animals.

The findings of Islam et al, (2025) support the importance of molecular diagnostics in detecting *Brucella* infections, particularly in seronegative animals. Meta-analysis of detection rates across studies showed significant variability ranging from 0.96% to 100%, highlighting differences in sample types, animal species, and regions. The pooled detection rate from random-effects models was 35.08%, indicating that many seronegative animals still carry *Brucella* species. Forest plot analysis further confirmed the heterogeneity in detection and emphasized the importance of using molecular detection alongside serological testing to identify latent carriers. These results underscore the limitations of antibody-based serological tests.

The current study revealed the potential cross-reactions between vaccine strains and wild strains. This finding aligns with the observations of Godfroid et al, (2010), who emphasized the value of indirect ELISAs in differentiating true infections from false positives and in distinguishing vaccination responses from natural infection. The current results similarly suggest that some serological tests may struggle to clearly differentiate these scenarios, particularly in the context of Rev1 and IRIBA, which may induce antibody responses overlapping with wild-type *Brucella* infection. In contrast, Pastor et al, (2006), and Ibarra et al, (2023) found that RBT, Wright, and 2ME-Wright produced consistently negative results in RB51-vaccinated animals across all sampling times, indicating minimal cross-reactivity. It indicated that RB51 does not induce antibodies against the O-side chain of lipopolysaccharide (LPS), thereby avoiding interference with commonly used serological tests such as the Rose Bengal, Wright and the 2ME-Wright test. This does not align with current findings; such

differences could be due to the use of difference in evaluated assays and tested animals. The current study evaluated the samples prepared form sheep animals which were vaccinated by REV1 antigens and did not receive RB51. The common antigens in *Brucella* strains could resulted in reactivity in ELISA assay.

The findings of Haro et al, (2025) further reinforce the utility of indirect ELISA (iELISA) in vaccinated and non-vaccinated populations. They reported that iELISA based on a synthetic antigen showed superior specificity (97–98%) compared to competitive ELISA (cELISA), while both tests had similar sensitivity (~94%). These results align with current findings that iELISA had high diagnostic value, especially in distinguishing negative cases by IRIBA antigen and positive case by using positive infected sheep. Supporting this, Cai et al. (2025) developed a highly specific and sensitive cELISA with no cross-reaction with other bacteria and excellent performance in both vaccinated and non-vaccinated animals. While current study did not include cELISA using *B. neotomae* LPS, the high sensitivity and specificity reinforce the potential of refined antigen selection in improving diagnostic performance. In a molecular perspective, Islam et al, (2025), underscored the advantage of molecular assays such as PCR and LAMP in detecting *Brucella* DNA in seronegative animals. Their meta-analysis highlighted substantial heterogeneity across studies but confirmed that molecular tools can detect latent infections missed by serology. Legesse et al, (2023), evaluated iELISA in African buffaloes and found that iELISA offered better repeatability and specificity. The current results similarly support the higher reliability and diagnostic value of antigen-based iELISA over other serological methods. The findings of the current study are consistent with those reported by Zhang et al, (2025) regarding the *Brucella suis* S2 vaccine in goats. In their study, the S2 vaccine was also shown to lack persistent pathogenic effects in the host, and the qPCR assay they developed successfully differentiated the vaccine strain from wild-type *Brucella* isolates. Both studies confirmed the absence of long-term persistence of the vaccine strain in vaccinated animals and highlighted the high safety profile of these vaccines. However, Zhang et al. (2025), reported the potential for cross-transmission from vaccinated to unvaccinated animals, either via aerosols or vertical transmission. Overall, this comparison suggests that live attenuated vaccines such as RB51 and S2 can play a valuable role in controlling brucellosis, particularly when implemented alongside specific diagnostic tools and appropriate herd management strategies (Zhang et al, 2025). In conclusion, current findings support the utility of indirect ELISA methods by using vaccine and wild type strains of *Brucella*, in accurately detecting *Brucella* infections. Continued refinement of antigen targets and integration of molecular tools are recommended to improve the accuracy and specificity of brucellosis diagnosis in both clinical and epidemiological settings.

In the current study, the Rose Bengal test demonstrated higher sensitivity compared to the Wright and 2ME-Wright tests, identifying more positive cases, although the agreement between tests was moderate (kappa=0.45 and 0.42). Although the current study did not directly assess vaccine efficacy, the lower S/P value of Rev1 (kappa=0.52; mean S/P=0.54±0.03) may suggest in its lower immunogenicity for humoral immune responses and lower interfering in serological diagnostic test. Poester et al, (2006) demonstrated that RB51 is safe even during late pregnancy. The findings of the current study indicate that the RB51 membrane antigen can be utilized for diagnosing negative sheep samples with high specificity in ELISA. This underscores the importance of selecting appropriate vaccine strains and diagnostic antigens to minimize false-

negative and false-positive results, thereby enhancing disease control strategies.

The current study highlights the diagnostic challenges in brucellosis-endemic regions, particularly in differentiating vaccine-induced antibodies from those arising due to natural infection. While conventional serological tests such as Rose Bengal, Wright, and 2ME remain widely used, their variable agreement and potential for false-positive results underscore the need for more refined tools. The indirect ELISA using vaccine and wild strains of *Brucella* showed superior sensitivity and specificity, suggesting its potential as a reliable diagnostic method in vaccinated populations. Implementing a combined diagnostic approach using serum agglutination test and ELISA using *Brucella* strains could improve diagnostic accuracy and reduce false results. Also, further comparative studies are warranted to evaluate the immunogenicity and diagnostic compatibility of different vaccine strains, including Rev1 and IRIBA under diverse field conditions. Longitudinal and multi-regional studies are needed to validate the using of *Brucella* strains in ELISA across different livestock.

Acknowledgments

The authors express their gratitude to the Razi Vaccine and Serum Research Institute and veterinary organizations for their collaboration in providing vaccines and serum samples.

Conflict of Interest

The authors declare that have no conflict of interest.

Funding

This study was financially supported by Shahid Chamran University of Ahvaz, Ahvaz, Iran, under grant number SCU.VP1403.12470.

References

Abdelgawad, H. A., Lian, Z., Yin, Y., Fang, T., Tian, M., & Yu, S. (2023). Characterization of Brucella abortus Mutant A19mut2, a Potential DIVA Vaccine Candidate with a Modification on Lipopolysaccharide. *Vaccines*, *11*(7), 1273.

Banyasz, B., Antal, J., & Denes, B. (2025). Ghostbuster—A Western Blot-Based Panel Method to Resolve False-Positive Brucellosis Serology Test Results. *Microorganisms*, *13*(3), 574.

Berhanu, G., & Pal, M. (2020). Brucellosis: A highly infectious zoonosis of public health and economic importance. *Journal of Emerging Environmental Technologies and Health Protection*, 3, 5-9.

Cai, G. H., Guo, C. Y., Guo, K. X., Zhang, J. D., Chen, H. C., & Liu, Z. F. (2025). Development of a competitive ELISA based on Brucella neotomae lipopolysaccharide for detecting brucellosis in livestock. *Analytical Biochemistry*, 115880.

Copin, R., Vitry, M. A., Hanot Mambres, D., Machelart, A., De Trez, C., Vanderwinden, J. M., ... & Muraille, E. (2012). In situ microscopy analysis reveals local innate immune response developed around *Brucella* infected cells in resistant and susceptible mice. *PLoS pathogens*, 8(3), e1002575.

Daro, B. S., Deneke, Y. A., & Negasa, J. G. (2024). Bovine Brucellosis: A Zoonotic Disease of Public Health and Economic Concern in Ethiopia. *Journal of Clinical Case Studies Reviews & Reports, 2* (2), 1-8.

Davis Jr, M. R., & Goldberg, J. B. (2012). Purification and visualization of lipopolysaccharide from Gram-negative bacteria by hot aqueous-phenol extraction. *Journal of visualized experiments: JoVE*, (63), 3916.

Dorneles, E. M., Lima, G. K., Teixeira-Carvalho, A., Araújo, M. S., Martins-Filho, O. A., Sriranganathan, N., ... & Lage, A. P. (2015). Immune response of calves vaccinated with Brucella abortus S19 or RB51 and revaccinated with RB51. *PloS one*, *10*(9), e0136696.

Falenski, A., Mayer-Scholl, A., Filter, M., Göllner, C., Appel, B., & Nöckler, K. (2011). Survival of Brucella spp. in mineral water, milk and yogurt. *International Journal of Food Microbiology*, *145*(1), 326-330.

Galinska, E. M., & Zagórski, J. (2013). Brucellosis in humans-etiology, diagnostics, clinical forms. *Annals of agricultural and environmental medicine*, *20*(2), 233-238.

Godfroid, J., Nielsen, K., & Saegerman, C. (2010). Diagnosis of brucellosis in livestock and wildlife. *Croatian medical journal*, *51*(4), 296-305.

Golding, B., Scott, D. E., Scharf, O., Huang, L. Y., Zaitseva, M., Lapham, C., ... & Golding, H. (2001). Immunity and protection against *Brucella abortus*. *Microbes and infection, 3*(1), 43-48.

Golshani, M., & Buozari, S. (2017). A review of brucellosis in Iran: epidemiology, risk factors, diagnosis, control, and prevention. *Iranian biomedical journal*, *21*(6), 349.

Haine, V., Sinon, A., Van Steen, F., Rousseau, S., Dozot, M., Lestrate, P., ... & De Bolle, X. (2005). Systematic Targeted Mutagenesis of *Brucella melitensis* 16M Reveals a Major Role for GntR Regulators in the Control of Virulence. *Infection and immunity*, *73*(9), 5578-5586.

Heidary, M., Dashtbin, S., Ghanavati, R., Mahdizade Ari, M., Bostanghadiri, N., Darbandi, A., ... & Talebi, M. (2022). Evaluation of brucellosis vaccines: a comprehensive review. *Frontiers in veterinary science*, 9, 925773.

Haro, A. D. G., Samaniego, M. Y. B., Moreno-Caballeros, P., Burbano-Enríquez, A., Torres, V. A. S., Mulki, M. C. G., ... & Saegerman, C. (2025). Bayesian Estimation of the True Bovine Brucellosis Prevalence in Vaccinated and Non-Vaccinated Ecuadorian Cattle Populations, and the Sensitivity and Specificity of a Competitive and Indirect ELISA Using a New Synthetic Antigen. *Microorganisms*, *13*(1), 69.

Ibarra, M., Campos, M., Hernan, B., Loor-Giler, A., Chamorro, A., & Nuñez, L. (2023). Comparison of diagnostic tests for detecting bovine brucellosis in animals vaccinated with S19 and RB51 strain vaccines. *Veterinary World*, *16*(10), 2080.

Ibarra, M., Campos, M., Ibarra, C., Gladys, U., Huera, D., Gutierrez, M., Nunez, L. (2023). Financial losses associated with bovine brucellosis (Brucella abortus) in carchi-ecuador. *Open Journal of Animal Sciences*, *13*(2), 205-216.

Idrees, H., Abbas, J., Raza, A., Qamar, M. H., Waheed, M. A., Arshad, M. B., ... & Raheem, A. (2024). A systematic overview of bovine brucellosis and its implications for public health: Bovine Brucellosis: A Zoonotic Threat. *Letters In Animal Biology*, 04(1), 01-09.

Islam, M. S., Habib, M. A., Tonu, N. S., Haque, M. S., & Rahman, M. M. (2025). Beyond Serology: A Meta-Analysis of Advancements in Molecular Detection of Brucella spp. in Seronegative Animals and Biological Samples. *Veterinary Medicine and Science*, *11*(1), e70200.

Jiao, H., Zhou, Z., Li, B., Xiao, Y., Li, M., Zeng, H., ... & Gu, G. (2021). The mechanism of facultative intracellular parasitism of Brucella. *International journal of molecular sciences*, *22*(7), 3673.

Keyvanfar, A., Sali, S., & Zamani, A. (2021). Epidemiology, clinical and laboratory manifestations, and outcomes of brucellosis among 104 patients in referral hospitals of Tehran, Iran. *Archives of Clinical Infectious Diseases*, *16*(2), e111546.

Khosravi, Mohammad. Ghorbanpour, Masoud. Fatemi Tabatabaei, Seyed Reza. Mohammad Alipour, Yasman. (2018). The effect of oral administration of betaine on the humoral immune response of mice to Brucella abortus. *Iranian Veterinary Journal*, *14*(1), 2018.

Kiros, A., Asgedom, H., & Abdi, R. D. (2016). A review on bovine brucellosis: Epidemiology, diagnosis and control options. *ARC Journal of Animal and Veterinary Sciences (AJAVS)*, *2*(3), 8-21.

Lalsiamthara, J., Gogia, N., Goswami, T. K., Singh, R. K., & Chaudhuri, P. (2015). Intermediate rough *Brucella abortus* S19Δper mutant is DIVA enable, safe to pregnant guinea pigs and confers protection to mice. *Vaccine*, *33*(22), 2577-2583.

Legesse, A., Mekuriaw, A., Gelaye, E., Abayneh, T., Getachew, B., Weldemedhin, W., Tesgera, T., Deresse, G. and Birhanu, K. (2023). Comparative evaluation of RBPT, I-ELISA, and CFT for the diagnosis of brucellosis and PCR detection of Brucella species from Ethiopian sheep, goats, and cattle sera. *BMC microbiology*, *23*(1), 216.

Li, Z. Q., Shi, J. X., Fu, W. D., Zhang, Y., Zhang, J., Wang, Z., ... & Zhang, H. (2015). A *Brucella melitensis* M5-90 wboA deletion strain is attenuated and enhances vaccine efficacy. *Molecular Immunology*, *66*(2), 276-283.

Lopez-Goni, I., Garcia-Yoldi, D., Marin, C. M., De Miguel, M. J., Munoz, P. M., Blasco, J. M., & Garin-Bastuji, B. (2008). Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all Brucella species, including the vaccine strains. *Journal of clinical microbiology, 46*(10), 3484-3487.

Markey, B., Leonard, F., Archambault, M., Culliname, A., and Maguire, D. (2013). Brucella species. Clinical Veterinary Microbiology. 2nd Ed. Elsevier, Toronto, Ontario, 325-334.

Martirosyan, A., & Gorvel, J. P. (2013). *Brucella* evasion of adaptive immunity. *Future microbiology*, *8*(2), 147-154.

Pal, M., Gizaw, F., Fekadu, G., Alemayehu, G., & Kandi, V. (2017). Public health and economic importance of bovine Brucellosis: an overview. *Am J Epidemiol*, *5*(2), 27-34.

Pandya, A. J., & Ghodke, K. M. (2007). Goat and sheep milk products other than cheeses and yoghurt. *Small Ruminant Research*, *68*(1-2), 193-206.

Perez, R. O., Mata, R. A., & Dominguez, G. A. (2024). Retrospective Brucellosis Study in Mexico. *International Journal of Veterinary Medicine*, *3*(1), 1-6.

Poester, F. P., Gonçalves, V. S., Paixao, T. A., Santos, R. L., Olsen, S. C., Schurig, G. G., & Lage, A. P. (2006). Efficacy of strain RB51 vaccine in heifers against experimental brucellosis. *Vaccine*, *24*(25), 5327-5334.

Poester, F. P., Nielsen, K., Samartino, L. E., & Yu, W. L. (2010). Diagnosis of brucellosis. *Open Veterinary Science Journal*, *4*(1), 46-60.

Priyantha, M. A. R. (2021). An overview of human brucellosis: a neglected zoonotic disease in livestock. *Sri Lanka Veterinary Journal*, *68*(1-2), 27-37.

Rehman, S., Ullah, S., Kholik, K., Munawaroh, M., Sukri, A., Malik, M. I. U., ... & Sucipto, T. H. (2025). A detailed review of bovine brucellosis. *Open Veterinary Journal*, *15*(4), 1520-1520.

Sadaqat, M. H. (2024). Pathogenesis, clinical manifestations, treatment and prevention of brucellosis in humans and animals. *Afghanistan Journal of Basic Medical Science*, *1*(1), 85-90.

Sadeghifard, N., & Pakzad, R. (2023). Investigation of epidemiological features of brucellosis in Iran: A systematic review and meta-analysis study. *Technology and Research Information System*, *5*(4), 0-0.

Smirnova, E. A., Vasin, A. V., Sandybaev, N. T., Klotchenko, S. A., Plotnikova, M. A., Chervyakova, O. V., ... & Kiselev, O. I. (2013). Current methods of human and animal brucellosis diagnostics. *Advances in Infectious Diseases*, *3*(3), 177-184.

Sofian, M., Aghakhani, A., Velayati, A. A., Banifazl, M., Eslamifar, A., & Ramezani, A. (2008). Risk factors for human brucellosis in Iran: a case–control study. *International journal of infectious diseases*, *12*(2), 157-161.

Stranahan, L. W., & Arenas-Gamboa, A. M. (2021). When the going gets rough: The significance of *Brucella* lipopolysaccharide phenotype in host–pathogen interactions. *Frontiers in Microbiology*, 12, 713157.

Whatmore, A. M. (2009). Current understanding of the genetic diversity of Brucella, an expanding genus of zoonotic pathogens. *Infection, Genetics and Evolution*, *9*(6), 1168-1184.

World Organization for Animal Health (OIE), (2021) Chapter 2.4.3; Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis*) (infection with *B. abortus*, *B. melitensis* and *B. suis*) (version adopted in May 2016). In: manual of diagnostic tests and vaccines for terrestrial animals 2021, 355-398.

Yaeger, M. J., Holler, L. D. (2007). Bacterial causes of bovine infertility and abortion. In *Current therapy in large animal theriogenology* (2nd ed) Chapter 49, Elsevier, St. Louis, 389-399.

Yang, Z., Chai, Z., Wang, X., Zhang, Z., Zhang, F., Kang, F., ... & Yue, J. (2024). Comparative genomic analysis provides insights into the genetic diversity and pathogenicity of the genus Brucella. *Frontiers in Microbiology*, 15, 1389859.

Yousefinejad, S., Khosravi, M., Gharibi, D., Mayahi, M., & Seyfi Abad Shapouri, M. R. (2024). Tracking the antibody level against Ornithobacterium rhinotracheal in the serum of slaughtered turkeys in Khuzestan province using In-house ELISA. *Iranian Veterinary Journal*, *20*(2), 128-138.

Zhang, H., et al. (2025). Establishment and application of a qPCR method for differential detection of Brucella S2 vaccine strain. *BMC Veterinary Research*, 21(1), 238.

Received: 28.06.2025
Accepted: 06.10.2025

مقاله کامل

دوره بیست و دوم ـ شماره ۱ ـ بهار ۱۴۰۵

DOI: 10.22055/ivj.2025.531580.2809

ارزیابی آنتی‌بادی‌های متقاطع در میان سویه‌های بروسلا: اعتبارسنجی آزمون‌های تشخیص سرمی بروسلوز

فرنگیس نرگسی[۱]، محمد خسروی[۲]*، داریوش غریبی[۳]، مهدی پورمهدی بروجنی[۴] و مسعود قربانپور[۵]

[۱] دانشجوی دکترای تخصصی باکتری‌شناسی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۲] دانشیار گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۳] استاد گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۴] استاد گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۵] استاد گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه شهرکرد، شهرکرد، ایران

تاریخ دریافت: ۱۴۰۴/۴/۷ تاریخ پذیرش: ۱۴۰۴/۷/۱۴

چکیده

هدف مطالعه حاضر بررسی واکنش‌های متقاطع آنتی‌بادی در سرم گوسفند نسبت به سویه‌های واکسن و پاتوژن بروسلا و همچنین اعتبارسنجی نتایج آگلوتیناسیون با بکارگیری آزمون الیزا و استفاده از هر دو سویه واکسن و وحشی بروسلا است. سویه‌های واکسن و وحشی استفاده شده شامل RB51، Rev1، B. melitensis و B. abortus بودند که همگی با آزمایش PCR تأیید شدند. در مجموع ۸۱ نمونه سرم گوسفند در این مطالعه گنجانده شدند که شامل نمونه‌های با واکنش مثبت و منفی در آزمون‌های آگلوتیناسیون بود. پس از ارزیابی سرم‌ها، آزمون‌های الیزا غیر مستقیم با استفاده از لیپو پلی‌ساکارید باکتری برای اعتبارسنجی نتایج آگلوتیناسیون و برای ارزیابی آماری واکنش‌های متقاطع سرم نسبت به سویه‌های واکسن و وحشی بروسلا به کار گرفته شد. از ۸۱ نمونه سرم مشکوک ارزیابی شده، آزمون رزبنگال ۶۱ نتیجه مثبت و ۲۰ نتیجه منفی را نشان داد. آزمون‌های رایت و 2ME رایت به ترتیب ۳۸ و ۳۶ نتیجه مثبت و ۴۳ و ۴۵ نتیجه منفی را نشان دادند. براساس تحلیل منحنی ROC، بالاترین مساحت زیر منحنی ۸۴ درصد برای *IRIBA*، ۷۵ درصد برای *Rev1*، ۷۰ درصد برای *B. melitensis* و ۶۸ درصد برای *B. abortus* بود. تحلیل آماری نشان داد که نوع آنتی‌ژن در آزمون‌های الیزا تأثیر معنی‌داری بر مقادیر S/P دارد. میانگین و خطای استاندارد مقادیر S/P برای واکسن Rev1 ۰/۵۴±۰/۳، *B. melitensis* ۰/۸۲±۰/۲، برای واکسن IRIBA ۰/۸۲±۰/۰۴ و آنتی‌ژن B. abortus ۰/۹۸±۰/۰۴ بود. بالاترین حساسیت و ویژگی با استفاده از آنتی‌ژن‌های *B. melitensis* و *IRIBA* به ترتیب به دست آمد. در الیزا غیرمستقیم که از هر دو نوع واکسن و سویه‌های وحشی بروسلا استفاده شد، حساسیت و ویژگی مناسبی نشان حاصل شد که بیان‌گر واکنش متقاطع سویه‌های مورد آزمون و همچنین، کاربرد آن به عنوان یک روش تشخیصی قابل اعتماد برای گوسفندهای واکسینه شده و آلوده است.

کلمات کلیدی: بروسلا، گوسفند، واکسن، واکنش متقاطع، الیزا

* نویسنده مسئول: محمد خسروی، دانشیار گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

E-mail: m.khosravi@scu.ac.ir

© 2020 by the authors. Licensee SCU, Ahvaz, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0 license) (http://creativecommons.org/licenses/by-nc/4.0/).