Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 7 (Special Issue): 772-779 (2023) (http://www.wildlife-biodiversity.com/)

Research Article

Enhancing Brucellosis diagnosis in Ovine populations: a comparative analysis of iELISA and RBPT in Thi-Qar province

Mokhtar H. Ramadhan*, Wasan A. Garbi

Institute of Genetic Engineering and Biotechnology for postgraduate studies, University of Baghdad, Iraq

*Email: mhrt1989@gmail.com

Received: 29 August 2023 / Revised: 15 November 2023 / Accepted: 25 November 2023/ Published online: 28 November 2023.

How to cite: Ramadhan, M.H., Garbi, W. (2023). Enhancing Brucellosis diagnosis in ovine populations: a comparative analysis of iELISA and RBPT in Thi-Qar province, Journal of Wildlife and Biodiversity, 7 (Special Issue), 772-779. **DOI**: https://doi.org/10.5281/zenodo.10384406

Abstract

In our scholarly endeavor at Oxford University, we meticulously investigated refined methodologies for detecting anti-brucella antibodies within the serum of ovine species. This study rigorously evaluates two predominant diagnostic approaches: the Rose Bengal Plate Test (RBPT) and the indirect Enzyme-Linked Immunosorbent Assay (iELISA). The RBPT, recognized for its expeditious nature, is a slide-based agglutination test employing antigens from *Brucella melitensis* biovar 1, specifically the Weybridge strain number 99. In contrast, iELISA represents an indirect detection paradigm, utilizing specific antigens to accurately identify antibodies within serum samples. Both diagnostic procedures commence with the standardization of serum samples and reagents to ambient temperature. This is followed by intricate protocols encompassing antigen-antibody interactions and meticulous observation of outcomes. The RBPT is characterized by its immediate visual agglutination, typically manifesting within minutes. Conversely, the iELISA approach discerns antibodies through a sequence of enzyme-mediated reactions, culminating in observable color variations. This comprehensive study encompassed the analysis of 259 serum samples extracted from female sheep, stratified across three distinct age brackets. Within this cohort, 37 out of 259 samples (14.29%) yielded positive results through RBPT, whereas a significantly larger proportion, 222 out of 259 (85.71%), were determined to be negative. In the realm of ELISA, 67 out of 259 samples (25.87%) were positively identified, leaving 192 out of 259 (74.13%) in the negative spectrum. These samples were meticulously collected from eight disparate fields in the Thi-Qar Province, located in the southern region of Iraq. The collection period spanned from November 2022 to June 2023, encompassing animals that exhibited clinical signs of the disease or had histories of abortion. Our findings lead to a compelling conclusion: the

ELISA technique demonstrates superior sensitivity and accuracy in detecting specific antibodies within physiological fluids when juxtaposed with the RBPT method. This revelation holds profound implications for the field of veterinary diagnostics, particularly in the context of brucellosis in sheep.
Keywords: *Brucella melitensis*, iELISA, Sheep, Serology, Diagnostic Techniques

Introduction

Brucellosis remains a serious zoonotic disease globally (Aloufi et al., 2016; El-Sayed & Awad, 2018; Facciolà et al., 2018). This condition poses substantial challenges in the realms of economics, veterinary science, and public health, particularly in developing nations. Its impact extends to various sectors, underscoring the need for effective management and control measures. The Enterobacteriaceae family, particularly Brucella melitensis, is associated with conditions like epididymitis, orchitis, and prostatitis, which may contribute to infertility in men (Al-Saadi & Abd, 2019; Beyene & Tesega, 2014; Franc et al., 2018). Controlling human brucellosis necessitates the eradication of the disease in animals (Franc et al., n.d.; Hamade et al., 2010). Br. melitensis not only affects sheep and goats but also causes miscarriages in cattle and undulating fever in humans (Al-Mossawy et al., 2019; Di Bonaventura et al., 2021; Sulaiman, 2017).

Currently, brucellosis is diagnosed using serological and microbiological methods, with varying sensitivities and specificities (Hill, 2005; Kim et al., 2007; Lopez et al., 2005). ELISA stands out as a highly sensitive and specific assay, with fewer false positives compared to agglutination tests (Merino et al., 1991; Xu et al., 2020). While culture is the most reliable diagnostic method, it is time-consuming and poses infection risks to technicians (de Jager et al., 2005; Smits et al., 2003). ELISA, due to its high sensitivity (89.1%) and specificity (98.2%), is suitable for quick field screening to detect IgM and IgG antibodies for brucellosis serodiagnosis in livestock (Abdoel et al., 2008; Alton et al., 1988).

Sample Collection

Blood samples were obtained from a total of 259 female sheep, categorized into three age groups: less than 1 year, 1-3 years, and over 3 years, as detailed in Tables 1 and 2. These samples were taken from eight different farms in the Thi-Qar province. The selection of these animals was based on their suspected infection status, derived from case histories and clinical observations. The serum samples, extracted from the collected blood, were then preserved at a temperature of -20°C until further analysis (Al-Naemi, 2010).

In addition to these samples, efforts were made to obtain specimens from sheep requiring isolation. However, challenges were faced in collecting samples from lymph nodes, bones, and chronic injury sites, which are known to harbor osteoarthritic bacterial infections. The complexity of these sites, including the spinal cord, further compounded the difficulty of sample collection. This study was conducted in collaboration with the Central Veterinary Laboratory.

Materials and methods

Indirect ELISA kits, provided by I.D Vet, Innovate Diagnosis Co., France, were employed as per the manufacturer's guidelines.

Rose Bengal Slide Agglutination Test (RBST)

The Rose Bengal Slide Agglutination Test, abbreviated as RBST, is a diagnostic procedure designed to identify antibodies specific to Brucella melitensis in various animal serums. This method utilizes a specialized agglutination mix containing Br. melitensis biovar 1 Weybridge strain number 99 antigens (supplied by RSA-RB, IDVet, France). Before initiating the test, both the serum samples and the antigen are equilibrated to room temperature within a biosafety cabinet. This is followed by thoroughly vortexing the contents. The next step involves combining 30 µl of the animal serum with an equal amount of antigen. This mixture is then placed on the shiny surface of a white tile. A sterile wooden stick is used for mixing. The tile is then gently rocked at room temperature for a duration of four minutes under ambient lighting conditions. During this period, the occurrence of agglutination is carefully monitored. Positive results, indicated by visible agglutination within this time frame, suggest the presence of an infection.

ELISA Assay Procedure

For the ELISA assay, reagents were first brought to room temperature (25˚C). Each strip used in the assay was labeled with a number. The ELISA test was performed following the guidelines provided by the manufacturer.

All procedures followed standard laboratory protocols and any modifications from previously published methods were minimal and necessary for the replication of this study. Detailed descriptions of these standard methods have been omitted, as they are widely known and referenced in the relevant scientific literature.

Table 1. Incidence of Brucellosis in Female Sheep Determined by RBPT Based on Age Group

Age Group (Years)	Number of Serum Samples Tested	Positive Cases	Incidence Rate (%)

Less than 1	68	4	5.81
1 to 3	105	20	19.04
More than 3	86	13	15.11
Total	259	37	14.29

Statistical Significance: $P \leq 0.05$

Table 2. Prevalence of Brucellosis in Female Sheep Assessed by iELISA Based on Age Group.

Age Group (Years)	Number of Serum Samples Tested	Positive Cases	Prevalence Rate (%)
Less than 1	68	8	11.76
1 to 3	105	28	26.66
More than 3	86	31	36.04
Total	259	67	25.88

Statistical Significance: $P \leq 0.05$

Results and Discussion

The semi-quantitative indirect ELISA technique and RBPT test revealed significant differences between the RBPT and ELISA in the tested samples. According to the ELISA cut-off point (S/P = 110-120% with Validation Criteria), samples with S/P% greater than or equal to 120% were considered positive, less than or equal to 110% were negative, and those between 110% and 120% were doubtful (Table 4).

In the study of 259 female sheep serum samples, 37/259 (14.29%) were positive by the RBPT test, while 222/259 (85.71%) were negative. In contrast, the ELISA technique showed that 67/259 (25.87%) were positive, and 192/259 (74.13%) were negative. The ELISA-positive samples varied in their S/P% values, ranging from 126% to 176%, with optical densities (OD) between 1.938 and 2.687 (Table 3 and Figure 3).

Table 3. iELISA Technique Data - Optical Density and Sample Percentage for 8 Samples

Result	S/P Ratio (%)	Optical Density (OD)	Well Location Controls	Reference
	0.071	A1	Negative Control	
	0.074	B1	Negative Control	
	1.561	C1	Positive Control	
	1.554	D1	Positive Control	
P	176%	2.687	F1	02
P	159%	2.437	E2	09
P	166%	2.534	B2	06
P	137%	2.108	D4	24
P	149%	2.285	B8	54
P	126%	1.938	F8	58

P	138%	2.118	E9	65
P	153%	2.338	C11	79

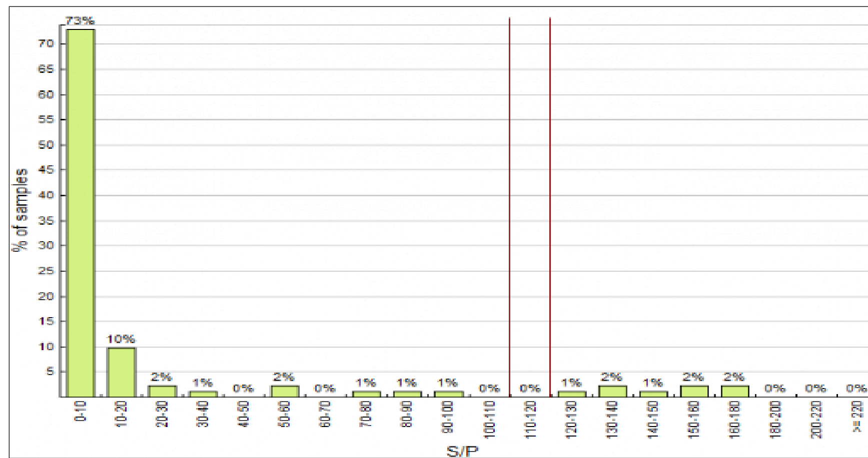

Figure 3. depicts the correlation between the percentage of samples tested and their respective Optical Density (OD) readings.

Table 4 provides a detailed presentation of the validation benchmarks for the ELISA (Enzyme-Linked Immunosorbent Assay) technique. A crucial metric for ascertaining the test's reliability is the ratio between the Optical Density of the Positive Control (ODPC) and the Optical Density of the Negative Control (ODNC). In this specific instance, achieving a ratio of 21.34 is considered a strong indicator of the test's validity. This high ratio underscores the significant difference in optical density readings between the positive and negative controls, which is essential for ensuring the accuracy and dependability of the ELISA method.

Table 4. Validation Criteria for the iELISA Technique

Validation Criteria	Details
Optical Density of Positive Control (ODPC)	> 0.35
Optical Density of Negative Control (ODNC)	0.073
Ratio (ODPC/ODNC)	21.34
Criteria Assessment	Valid

The table meticulously details the key validation metrics for the indirect Enzyme-Linked Immunosorbent Assay (iELISA) method. It encompasses the Optical Densities (ODs) of both the positive and negative controls, and critically, the calculated ratio derived from these ODs. This ratio is fundamental in affirming the assay's credibility**.**

Upon analyzing the data, it was observed that of the 67 samples testing positive via ELISA, 30

instances (44.77%) were categorized as mild titer infections, and the remaining 37 (55.22%) as moderate to severe infections. This differentiation is crucial for understanding the infection's severity and guiding appropriate treatment strategies**.**

To compute and interpret the results of iELISA, a comparative analysis of the OD values from the negative control and serum samples against the OD of the positive control is performed. The formula for calculating the Sample-to-Positive percentage (S/P%) for each sample is delineated as follows**:**

S/P%=(OD of sample−OD of negative controlOD of positive control−OD of negative control)×100S/P%=(OD of positive control−OD of negative controlOD of sample−OD of negative control)x **100**

This calculation is pivotal in quantifying the presence and intensity of the targeted antibodies within the sample**.**

The validation criteria set for the test are stringent to ensure accuracy and reliability. These criteria encompass:

1. The average OD of the positive control (ODpc) must be greater than 0.350.
2. The ratio of the mean OD values for positive and negative controls (ODpc and ODnc) should exceed 3 (ODpc/ODnc > 3).

For individual serum or plasma samples, considering short or overnight incubation, the interpretation is as follows:

- Samples with an S/P% less than or equal to 110% are classified as negative.
- Samples with an S/P% greater than 110% but less than 120% are considered doubtful.
- Samples with an S/P% greater than or equal to 120% are deemed positive.

The findings indicate that a significant proportion of the positive sheep might have been previously or recently exposed to brucellosis (Krueger et al., 2014). The impact of seasonal climate variations on sheep health, particularly regarding brucellosis susceptibility, is also noteworthy (Al-Bayatti, 2006). The study's results showed that 32 of the 67 ELISA-positive sheep had experienced abortions, primarily in the older age groups, suggesting a correlation between age and infection prevalence (Faik, 2013).

It is also evident that sexually mature sheep (ages 1-3 years and over 3 years) are more susceptible to brucellosis than younger sheep (Hade, 2014). This increased susceptibility could be due to the presence of erythritol in reproductive fluids, which facilitates the multiplication of Brucella bacteria (Rahawy & AL-Timimi, 2016).

The interpretation of the ELISA results, including the calculation of S/P ratios and their categorization into negative, doubtful, or positive, is critical for accurate diagnosis (Table 5).

Table 5. Interpretation of S/P Percentage Results in iELISA

Result Range	Status
S/P % less than 110%	Negative
110% < S/P % < 120%	Doubtful
S/P % greater or equal to 120%	Positive

This table categorizes the outcomes of the iELISA test based on the calculated S/P percentage, defining the thresholds for negative, doubtful, and positive results.

Statistically, the data was analyzed using variance analysis for virulence genes values and independent t-tests, with significance set at a 5% level (Connelly, 2019). The frequency data was processed using Pearson's chi-squared test and Fisher's exact test, employing SPSS v26 (2019) for data analysis.

References

Abdoel, T., Dias, I. T., Cardoso, R., & Smits, H. L. (2008). Simple and rapid field tests for brucellosis in livestock. *Veterinary Microbiology*, *130*(3–4), 312–319.

Al-Bayatti, S. A. (2006). BACTERIOLOGICAL & SEROLOGICAL STUDY OF BRUCELLOSIS IN BUFFALO IN BAGHDAD. *The Iraqi Journal of Veterinary Medicine*, *30*(2), 23–30.

Al-Mossawy, F. K., Mohammed, N. S., & Al-Zubaidy, I. A. (2019). Determination of Brucella. abortus Biovars infected human in the middle and southern of iraq. *Journal of Biotechnology Research Center*, *13*(2), 17–25.

Al-Naemi, E. (2010). STUDYING THE HISTOPATHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF CRUD AND PARTIALLY PURIFIED LIPOPOLYSACCARID FROM Brucella abortus (BIO VAR6) ON LAB MICE. *Iraqi Journal of Biotechnology*, *9*(3).

Al-Saadi, B. Q. H., & Abd, A. S. (2019). The effect of bacterial infection on male infertility. *Iraqi Journal of Biotechnology*, *18*(3).

Aloufi, A. D., Memish, Z. A., Assiri, A. M., & McNabb, S. J. N. (2016). Trends of reported human cases of brucellosis, Kingdom of Saudi Arabia, 2004–2012. *Journal of Epidemiology and Global Health*, *6*(1), 11–18.

Alton, G. G., Jones, L. M., Angus, R. D., & Verger, J. M. (1988). *Techniques for the brucellosis laboratory.* Institut National de la recherche Agronomique (INRA).

Beyene, T., & Tesega, B. (2014). Rational veterinary drug use: Its significance in public health. *Journal of Veterinary Medicine and Animal Health*, *6*(12), 302–308.

Connelly, L. (2019). Chi-square test. *Medsurg Nursing*, *28*(2), 127.

de Jager, W., Prakken, B. J., Bijlsma, J. W. J., Kuis, W., & Rijkers, G. T. (2005). Improved multiplex immunoassay performance in human plasma and synovial fluid following removal of interfering heterophilic antibodies. *Journal of Immunological Methods*, *300*(1–2), 124–135.

Di Bonaventura, G., Angeletti, S., Ianni, A., Petitti, T., & Gherardi, G. (2021). Microbiological laboratory diagnosis of human brucellosis: An overview. *Pathogens*, *10*(12), 1623.

El-Sayed, A., & Awad, W. (2018). Brucellosis: Evolution and expected comeback. *International Journal of Veterinary Science and Medicine*, *6*, S31–S35.

Facciolà, A., Palamara, M. A. R., D'Andrea, G., Marano, F., Magliarditi, D., Puglisi, G., Picerno, I., Di Pietro, A., & Visalli, G. (2018). Brucellosis is a public health problem in southern Italy: Burden and epidemiological trend of human and animal disease. *Journal of Infection and Public Health*, *11*(6), 861–866.

Faik, A. J. (2013). Molecular Diagnosis of Brucella species in Baghdad. *Baghdad Science Journal*, *10*, 1.

Franc, K. A., Krecek, R. C., Haesler, B., & Arenas-Gamboa, A. M. (n.d.). Brucellosis remains a neglected disease inthe developing world: a call forinterdisciplinary action. *BMC Public Health*, *18*(125).

Franc, K. A., Krecek, R. C., Häsler, B. N., & Arenas-Gamboa, A. M. (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health*, *18*(1), 1–9.

Hade, B. F. (2014). Molecular Detection to Toxoplasma gondii in Serum Sheep Samples. *Iraqi Journal of Biotechnology*, *13*(2), 58–65.

Hamade, K. H., Jasem, K. A., & Abood, Z. H. (2010). Comparison between the efficiency of serological tests for Identification of Brucellosis. *Baghdad Science Journal*, *7*(2).

Hill, I. D. (2005). What are the sensitivity and specificity of serologic tests for celiac disease? Do sensitivity and specificity vary in different populations? *Gastroenterology*, *128*(4), S25–S32.

Kim, J. W., Lee, Y. J., Han, M. Y., Bae, D. H., Jung, S. C., Oh, J. S., Ha, G. W., & Cho, B. K. (2007). Evaluation of immunochromatographic assay for serodiagnosis of Brucella canis. *Journal of Veterinary Medical Science*, *69*(11), 1103–1107.

Krueger, W. S., Lucero, N. E., Brower, A., Heil, G. L., & Gray, G. C. (2014). Evidence for unapparent Brucella canis infections among adults with occupational exposure to dogs. *Zoonoses and Public Health*, *61*(7), 509–518.

Lopez, G., Ayala, S. M., Escobar, G. I., & Lucero, N. E. (2005). Use of Brucella canis antigen for detection of ovine serum antibodies against Brucella ovis. *Veterinary Microbiology*, *105*(3–4), 181–187.

Merino, A. L., Santiago, R. L., Ocampo, D. A., Monroy, I. H., & Dominguez, F. G. (1991). Brucelosis: avances y perspectivas. In *Brucelosis: avances y perspectivas* (p. 54).

Rahawy, M. A., & AL-Timimi, I. H. (2016). Analysis of Polymorphism of Melatonin Receptor Type 1A (MTNR1A) Gene, in Iraqi Local Sheep Using PCR-RFLP Technique. *Iraqi Journal of Biotechnology*, *15*(3).

Smits, H. L., Abdoel, T. H., Solera, J., Clavijo, E., & Diaz, R. (2003). Immunochromatographic Brucella-specific immunoglobulin M and G lateral flow assays for rapid serodiagnosis of human brucellosis. *Clinical and Vaccine Immunology*, *10*(6), 1141–1146.

Sulaiman, K. M. (2017). Chromosomal study for Assessment of Recurrent Spontaneous Miscarriage by Fluorescence in situ Hybridization (FISH) technique in Erbil City Iraqi-Kurdistan Region. *Iraqi Journal of Cancer and Medical Genetics*, *10*(2).

Xu, N., Wang, W., Chen, F., Li, W., & Wang, G. (2020). ELISA is superior to bacterial culture and agglutination test in the diagnosis of brucellosis in an endemic area in China. *BMC Infectious Diseases*, *20*(1), 1–7.