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Analytical performance evaluation of a rapid immunochromatographic test for the diagnosis of bovine brucellosis based on a recombinant BP26 protein

[*Avaliação do desempenho analítico de um teste imunocromatográfico rápido para o diagnóstico da brucelose bovina baseado em uma proteína recombinante BP26*]

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ABSTRACT

Brucellosis is an important bacterial disease of global distribution with zoonotic potencial. Serological tests used in Brazil for diagnosis of bovine brucellosis, including the Rose Bengal test (RBT), 2-mercaptoethanol (2-ME), fluorescent polarization (FPA), and complement fixation (FC), are based on the smooth lipopolysaccharide antigen (S-LPS) of *Brucella abortus*. The aim of this study was to evaluate a recombinant BP26 protein used as antigen in a rapid lateral flow immunochromatographic assay (rBP26-LFIA) for serological diagnosis of bovine brucellosis. Analytical performance of rBP26-LFIA was evaluated in positive and negative bovine serum samples previously characterized by RBT and 2-ME. Estimates of analytical sensitivity and specificity were 73.91% and 97.14%, respectively. Bovine sera reactive to *Neospora*, *Trypanosoma vivax* or *Leptospira* were used to assess specificity because these diseases are commonly associated with abortion in cattle. In addition to a possible cross-reaction induced by commercial vaccines against *Leptospira* in serological tests for bovine brucellosis using S-LPS as an antigen. In conclusion, rBP26-LFIA, with its current standardization, had good analytical performance. However, a future evaluation of diagnostic performance by rBP26-LFIA with samples from regions with known prevalence is necessary for its recommendation for use in the Brazilian program for the control and eradication of bovine brucellosis.

Keywords: *Brucella abortus*, BP26, serology, diagnosis

RESUMO

A brucelose é uma importante zoonose de proporções mundiais. Os testes sorológicos utilizados no Brasil, antígeno acidificado tamponado (RBT), soroaglutinação lenta/2-mercaptoetanol (2-ME), polarização fluorescente (FPA) e fixação de complemento (FC), são baseados no antígeno lipopolissacarídeo liso (S-LPS) da Brucella abortus*. O objetivo deste estudo foi avaliar uma proteína BP26 recombinante utilizada como antígeno em um teste rápido imunocromatográfico de fluxo lateral (rBP26-LFIA) para o diagnóstico sorológico da brucelose bovina. As características de desempenho analítico foram avaliadas em amostras de soro bovino positivas e negativas previamente caracterizadas pelos testes RBT e 2-ME. As estimativas da sensibilidade e especificidade analíticas foram de 73,91% e 97,14%, respectivamente. Soros bovinos reagentes à* Neospora, Trypanossoma vivax *e* Leptospira *foram utilizados para avaliação de especificidade, em razão de essas doenças serem causas frequentes de aborto em bovinos, além de ser possível reação cruzada induzida por vacinas comerciais contra Leptospira, em testes sorológicos para a brucelose bovina que utilizam S-LPS como antígeno. Como conclusão, o teste rBP26-LFIA, com sua atual padronização, apresentou um bom desempenho analítico. Contudo, uma futura avaliação do desempenho diagnóstico do teste rBP26-LFIA com amostras provenientes de regiões com prevalência conhecidas é necessária para sua recomendação de uso no programa brasileiro de controle e erradicação da brucelose bovina.*

Palavras-chave: Brucella abortus, *BP26, sorologia, diagnóstico*

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INTRODUCTION

Brucellosis is a chronic infectious disease caused by facultative intracellular bacteria of the genus *Brucella*, which affect various animal species, as well humans (Corbel, 2006). In Brazil, bovine brucellosis caused by *Brucella abortus* is enzootic with prevalences ranging from 0.91% to 30.6% in many Brazilian states (Brazil, 2020).

Serological diagnostic tests to detect bovine brucellosis that are used in Brazil according to the adopted by the Brazilian Program for Animal Brucellosis and Tuberculosis Control and Eradication – PNCEBT (Brazil, 2020) includes the rose Bengal test (RBT) as a screening test, and the 2-mercaptoethanol plus standard tube agglutination test (2-ME) or the complement fixation test (CF) as confirmatory tests (Meirelles-Bartoli and Mathias, 2010). Importantly, these tests are based on the detection of antibodies against the smooth lipopolysaccharide (S-LPS) antigen of *Brucella* spp., more precisely the O chain. However, this antigen despite being immunodominant and conserved in the smooth species of *Brucella* spp., it cannot differentiate naturally infected cattle from those vaccinated with the B19 strain. In addition, this antigen may result in cross-reactions with other gram-negative bacteria that have a similar LPS structure, leading to wrong decision-making regarding segregation and culling of non-infected animals (Poester *et al*., 2010).

Antigens other than S-LPS, have been characterized and studied for their antigenic potential associated with a variety of types of serological tests (Bai *et al*., 2021). Among these, the *Brucella* periplasmic protein 26 (BP26) stands out. This protein was identified as immunoreactive in animals infected with *Brucella* spp. and studied as a promising antigen to prevent false-positive results since it induces antibody production in a significant proportion of infected animals and does not show cross-reactivity with other gram-negative bacteria (Ducrotoy *et al*., 2016). The BP26 protein was used as an antigen in an enzyme-linked immunosorbent assay (ELISA) for bovine brucellosis with analytical sensitivity and specificity of 100% (25/25) and 94% (47/50), respectively, idicating the potential of this antigen for a brucellosis diagnostic test (Kumar *et al*., 2008). In the diagnosis of brucellosis, rapid tests are being developed in various parts of the world, especially in countries with limited infrastructure and financial resources (Hobbs *et al*., 2021).

All rapid lateral flow immunochromatographic assays (LFIA) so far developed and evaluated in the world were based on the use of *B. abortus* S-LPS as an antigen, with similar sensitivity and specificity to classic serological tests for bovine brucellosis (Tiwari *et al*., 2011; Manat *et al*., 2016; Bulashev *et al*., 2019; Gusi *et al*., 2019).

This study aimed to evaluate the analytical performance of a rapid test using a recombinant BP26 protein as an antigen (rBP26-LFIA) considering its potential as a point-of-care screening diagnostic test for bovine brucellosis.

MATERIAL AND METHODS

Serum samples employed in this study was obtained from archives of diagnostic samples, with the exeption of calves vaccinated with the RB51 strain and their controls, which was generated by an experimental protocol previously approved by the Institutional Animal Care and Use Committee (CEUA-UFMG protocol number 365/2018).

Twenth-three positive bovine serum samples from the Laboratório Federal de Defesa Agropecuária (LFDA/MG) in Minas Gerais were used to evaluate the analytical sensitivity (ASe) of the rBP26-LFIA test, one reference serum (CSLB000442) reagent to RBTand 2-ME confirmed by isolation of *B. abortus* and 22 bovine serum samples positive for brucellosis reactive to RBT and 2-ME without isolation. Of the 22 positive serum samples, two weak and strong positive serum samples by RBT and 2-ME, CSLB000002 and CSLB000093, respectively, were used (Table 1).

To evaluate the analytical specificity (ASp), a sample of bovine serum (CSLB 000073) from the LFDA/MG negative in RBT and 2-ME, 10 RBT non-reactive bovine serum samples from the state of Santa Catarina and from the Escola de Veterinária of the Universidade Federal de Minas Gerais and 11, 13 and 15 bovine serum samples reactive for *Neospora*, *Trypanosoma vivax*, and *Leptospira*, respectively, but not

reactive for RBT. In addition, we used 20 serum samples from calves aged less than 8 months that were vaccinated with RB51 (n = 10) or inoculated with sterile fosfate buffered saline (PBS) and non-reactive to RBT (n = 10). Serum samples from calves were collected and evaluated on day 0, 45, and 90 days after immunization with RB51 or inoculation with sterile PBS (Table 2).

The recombinant BP26 protein (rBP26) used in the rapid test of this project was developed by França *et al*. (2014). In summary, cloning for the production of rBP26 used the pXT7 plasmid (containing a kanamycin cassette) and the specific sequences of the BMEI0536 gene located on chromosome 1 of *B. melitensis* (GenBank accession number AE008917.1). A homologous recombination of the insert obtained with chemically immunocompetent *Escherichia coli* BL21 was performed. The rBP26 generated was composed of 250 amino acids with a histidine tail (six residues) and a molecular mass of 26.56 kDa.

The rapid test for brucellosis evaluated in this study was based on strip chromatography developed by Safetest Diagnostics, Belo Horizonte, Minas Gerais. It is a serological test for the detection of bovine immunoglobulins of isotypes A, G and M, called Pan-Ig. Briefly, first, a passive conjugation of colloidal gold with the recombinant protein BP26 (rBP26) was prepared. At the same time, the colloidal gold was conjugated with the mouse IgG antibody, allowing the visualization of the control band of the test. After conjugation of colloidal gold with rBP26 and mouse IgG, both were impregnated in a glass fiber using an impregnator (ClaremontBio) to obtain the conjugate membrane (conjugate pad). The rBP26 and the anti-mouse IgG antibody were also impregnated on a nitrocellulose membrane in the test and control lines, respectively. Subsequently, the test sheet (uncut sheet) with the following parts: conjugate membrane (conjugate pad), nitrocellulose membrane, absorbent membrane (absorbent pad) and membrane for sample application (sample pad) were assembled. The uncutsheet was taken to the automatic guillotine (A point) to cut the strips to a size of 0.31 cm. After cutting, the tapes were fitted into the base of a plastic cassette and then the cassette lid was fitted. For the correct closure of the cassettes, a conveyor belt containing a compressor roller was used. In the next step, the cassettes were placed individually in a laminated bag along with silica and sealed using an automatic sealer (Cetro). Then, the bags containing the cassettes were placed in a secondary box along with a bottle of buffer solution. All reagents were stored at a temperature of 24°C (± 3°C) until use.

To perform the test, sera were thawed at room temperature. Briefly, the cassettes were placed on a flat surface followed by the addition of 10 µL of undiluted serum and 150 µL of running buffer (phosphate buffer saline with bovine serum albumin). After 20 minutes of incubation at room temperature (22°C to 25°C), the interpretation of the results was carried out through visual observation of the expected sample bands and the test control. Bands viewed after that time were not considered. The test was considered negative if only the test control band appeared. If a second band appeared in the test area, the result was interpreted as positive (Figure 1). If the test control band did not appear, the test was considered invalid.

Data were organized into contingency tables for estimates of ASe, ASp, and accuracy relative to the official RBT and 2-ME tests. The performance parameters (ASe and ASp) were estimated using the MedCalc Statistical Software version 20.215 program, with a 95% confidence interval. Accuracy was calculated considering the sum of positive and negative values in both tests divided by the total number of samples multiplied by 100.

RESULTS

The reference serum CSLB000442 undiluted or diluted at 1:2 and 1:4 was positive by the rBP26-LFIA test (Table 1). All serial dilutions, with the exception of 1:2 and 1:4 were negative by the rBP26-LFIA test (Tab. 1), whereas all dilutions of CSLB000442 were positive by RBT and 2-ME. The CSLB000002 serum characterized as weakly positive by RBT (reaction strength +1) was negative by rBP26-LFIA. Sera CSLB000093 was correctly classified by rBP26-LFIA as positive (Tab.1). Of the 20 serum samples positive in RBT and 2-ME, 15 reacted as positive in rBP26-LFIA.The control band of rBP26-LFIA was reactive in all tests performed.

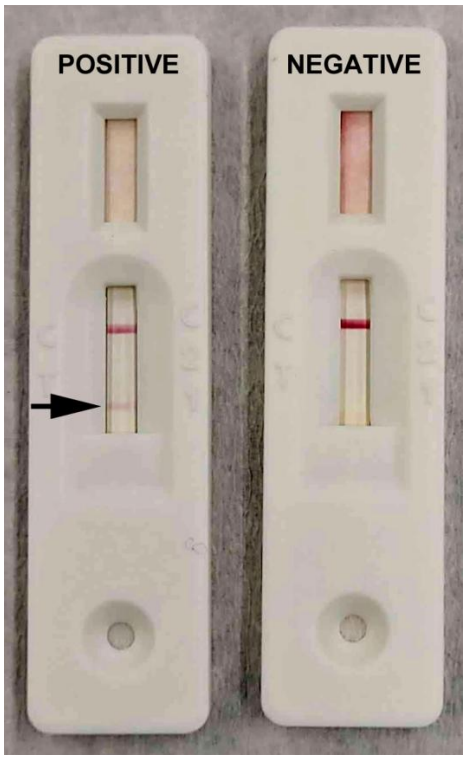

Figure 1. Representative results of the rapid test using a recombinant BP26 protein as an antigen (rBP26-LFIA), On the left a positive test with a contol band (top) and the test band (bottom - arrow). On the right, a negative test with a single control band visible.

Table 1. Results of the analytical sensitivity (ASe) evaluation of rBP26-LFIA

Serum ID/diluition	rBP26-LFIA	RBT/SAL/2-ME
CSLB000442	Positive	Positive
CSLB000442 1:2	Positive	Positive
CSLB000442 1:4	Positive	Positive
CSLB000442 1:8	Negative	Positive
CSLB000442 1:16	Negative	Positive
CSLB000442 1:32	Negative	Positive
CSLB000442 1:64	Negative	Positive
CSLB000442 1:128	Negative	Positive
CSLB000442 1:5	Negative	Positive
CSLB000442 1:10	Negative	Positive
CSLB000442 1:20	Negative	Positive
CSLB000442 1:40	Negative	Positive
CSLB000442 1:80	Negative	Positive
CSLB000442 1:25	Negative	Positive
CSLB000442 1:50	Negative	Positive
CSLB000442 1:100	Negative	Positive
CSLB000002	Negative	Positive
CSLB000093	Positive	Positive
20 LFDA/MG positive sera	15/20 Positive	Positive
ASe (23 sera)		17/23 (73.91%)
CI 95%		(51.59% - 89.77%)
Accuracy		73.91%

ASe: analytical sensitivity 95% CI: 95% confidence interval.
CSLB000442: positive reference serum with isolation of *B. abortus*.
LFDA/MG: Laboratório Federal de Defesa Agropecuária.

The serum CSLB000073 was correctly classified as negative by rBP26-LFIA. All 10 sera negative for brucellosis in RBT were also negative by rBP26-LFIA. One out of 11 serum samples reagent for *Neospora* was positive by rBP26-LFIA (Table 2). All 13 sera reagent for *Trypanosoma vivax*, and all 15 sera reagent for *Leptospira* were negative by rBP26-LFIA. None of these sera was RBT reactive. Of the 10 serum samples from calves immunized with the commercial RB51 vaccine, only one (1/10) was positive on days 45 and 90 after immunization by rBP26-LFIA. None of the animals inoculated with PBS were positive by rBP26-LFIA on the different days after inoculation (Table 2).

According to the results obtained, the analytical sensitivity was estimated at 73.91% (95% CI: 51.59% - 89.77%) and the analytical specificity at 97.14% (95% CI: 90.06% - 99.65%), correponding to 91,4% overall analitical accuracy. The estimated analytical accuracy for sensitivity and specificity was 73.91% and 97.14%, respectively (Table 1 and 2).

Table 2. Results of the analytical specificity (ASp) evaluation of the rBP26-LFIA.

serum ID	RBT	SAL/2-ME	total sera	Non-reagent sera in rBP26-LFIA		
CSLB000073	Negative	Negative	1	1/1		
Negative sera for brucellosis (field)	Negative	Not done	10	10/10		
Neospora reagent sera	Negative	Not done	11	10/11		
Trypanosomavivax reagent sera	Negative	Not done	13	13/13		
Leptospira reagent sera	Negative	Not done	15	15/15		
	RBT	SAL/2-ME	total sera	D0	D45	D90
Calves immunized with RB51	Negative	Not done	10	10/10	9/10	9/10
Calves inoculated with PBS	Negative	Not done	10	10/10	10/10	10/10
Total			70			
ASp (70 serums)				68/70 (97.14%)		
CI 95%				(90.06% - 99.65%)		
Accuracy				97.14%		

CI: 95% confidence interval.
D 0, D 45 and D 90: days after immunization.
RBT: Rose Bengal test; SAL: slow seroagglutination; 2-ME: 2-mercaptoethanol.
CSLB: LFDA/MG serum collection for bovine brucellosis.

DISCUSSION

The rBP26-LFIA was able to detect antibodies against the rBP26 protein in a dilution of up to 1:4 of a known positive serum (CSLB000442) from a cow reactive to the RBT and 2-ME tests and with isolation of *B. abortus*. Comparing the reaction of the CLSB000442 serum with the diagnostic tests that have S-LPS as antigen (RBT/2-ME), positive reactions were obtained in higher dilutions than those obtained with the rBP26-LFIA. These results agree with the literature since S-LPS is immunodominant in relation to other bacterial antigens (Cloeckaert *et al*., 2002), although several authors consider BP26 as one of the immunodominant proteins in the immune response of animals infected with *B. abortus* (Ducrotoy *et al*., 2016). BP26 has been previously used as antigen for serologic diagnosis of brucellosis (Kumar *et al*., 2008; França *et al*., 2014). However, its function is not completely understood, but it has been demonstrated the BP26 can form octamers that generate a channel-like structure (Kim *et al*., 2013).

Even though analytical sensitivity of rBP26-LFIA was lower compared to the RBT and 2-ME

tests, this type of test has the advantage of being fast and can be carried out in the field, without the need for sample preparation, laboratory installation and specific technical training. It can be an alternative for use in places with low infrastructure, restricted resources and in animal populations located in hardly accessible regions. The use of these types of tests allows control measures to be carried out more quickly, thus avoiding the spread of the disease within the herds.

Analytical specificity was evaluated against *Neospora caninum* and *Leptospira*, which are common causes of abortion in cattle (Silva *et al*, 2009). Cross-reaction induced by commercial vaccines against *Leptospira* spp. in serological tests for bovine brucellosis that use S-LPS as an antigen has been reported (Faria Naves *et al*., 2012). However, in the results of the analytical specificity of rBP26-LFIA in this study, no cross reactions were observed with the rBP26-LFIA. One of the samples from cattle infected with *Neospora* yielded a positive result by rBP26-LFIA, although there are no previous reports of cross-reactivity between BP26 or *Brucella* antigens with anti-*Neospora* antibodies.

The RB51 *B. abortus* vaccine strain with rough LPS that lacks the LPS O chain. Thus, it is possible that other antigens of the RB51 vaccine strain, such as the BP26 (OMP28) protein, may become accessible to the host's immune system, leading to the detection of a reaction in the rBP26-LFIA. On day 0 after immunization of calves with RB51, all 10 serum samples were negative in the rBP26-LFIA, in agreement with the RBT result. However, 45 and 90 days after immunization with RB51, a serum sample reacted in the rBP26-LFIA, which may be related to the delayed production of antibodies to this type of antigen, as already mentioned by Ducrotoy *et al*., 2016. According to the work by Guci *et al*., 2019, who evaluated the performance of three LPS-based diagnostic tests (RBT, iELISA and LFIA) for bovine brucellosis, only the RBT test did not react with sera from animals vaccinated with RB51. According to the literature, the type of test such as ELISA and LFIA can expose the most internal antigens of the bacteria, facilitating the development of responses to antigens other than LPS (Ducrotoy *et al*., 2016).

In the LFIA evaluated by Abdoel *et al*. (2008), Elshemey e Abd-Elrahman (2014), Herrera *et al*. (2015), and Gusi *et al*. (2019) using *B. abortus* S-LPS as antigen, the specificity estimates were slightly better than the sensitivity, with little difference with the conventional tests (RBT, FC or iELISA) used for comparison. The estimate of the analytical specificity of the test evaluated in this study was also better in relation to its analytical sensitivity, suggesting a possible direction of this test for use in the context of false-positive animals or low-prevalence for brucellosis.

The results found in the literature on the use of the BP26 protein as antigen were all developed in an ELISA, which presented good performance evaluations compared to traditional tests. All authors used samples characterized by imperfect tests or poorly characterized convenience samples that can lead to overestimated performance parameters and differences in estimates between authors (Kumar *et al*., 2008; Tiwari *et al*., 2011; Manat *et al*., 2016; Bulashev *et al*., 2019; Tian *et al*., 2020; Bai *et al*., 2021). Conversely, all previously developed LFIA used S-LPS as antigen unlike this study (Abdoel *et al*., 2008; Elshemey and Abd-Elrahman, 2014; Herrera *et al*., 2015; Gusi *et al*., 2019). These conditions limit the comparison of results obtained in the present study with the others, since this is the first study that evaluates the use of the antigen based on the BP26 protein in a LFIA for the serological diagnosis of bovine brucellosis.

CONCLUSION

The rBP26-LFIA, with its current standardization, showed good analytical performance. However, a future evaluation of the diagnostic performance of the rBP26-LFIA test with representative samples from regions with known prevalence is necessary for its recommendation for use in the Brazilian program for the control and eradication of bovine brucellosis as potential a point-of-care screening test.

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REFERENCES

ABDOEL, T.; DIAS, I.T.; CARDOSO, R. *et al*. Simple and rapid field tests for brucellosis in livestock. *Vet. Microbiol*., v.130, p.312-319, 2008.

BAI, Q.; LI, H.; WU, X. *et al*. Comparative analysis of the main outer membrane proteins of *Brucella* in the diagnosis of brucellosis. *Biochem. Biophys. Res. Commun*., v.560, p.126-131, 2021.

BRAZIL. Ministry of Agriculture, Livestock and Supply. Situational diagnosis of the PNCEBT. Brasilia: 2020. 102 p. Available in: DSPNCEBT.pdf (www.gov.br). Accessed in: 24 Jun. 2021.

BULASHEV, A.; AKIBEKOV, O.; SURANSHIYEV, Z. *et al*. Serodiagnostic potential of *Brucella* outer membrane and periplasmic proteins. *Turk. J. Vet. Anim. Sci*., v.43, p.486-493, 2019.

CLOECKAERT, A.; VIZCAINO, N.; PAQUET, J.Y. *et al*. Major outer membrane proteins of *Brucella* spp.: past, present and future. *Vet. Microbiol*., v.90, p.229-247, 2002.

CORBEL, M.J. *Brucellosis in humans and animals*. Geneva: WOAH, 2006. 89p.

DUCROTOY, M.J.; CONDE-ÁLVAREZ, R.; BLASCO, J.M. *et al*. A review of the basis of the immunological diagnosis of ruminant brucellosis. *Vet. Immunol. Immunopathol*., v.171, p.81-102, 2016.

ELSHEMEY, T.; ABD-ELRAHMAN, A. Evaluation of a rapid immunochromatographic test for detection of *Brucella abortus* antibodies in Egyptian cattle sera and milk. *Alexandria J. Vet. Sci*., v.40, p.24, 2014.

FARIA NAVES, J.H.F.; REZENDE, L.M.; RAMOS, G.C. *et al*. Interference in diagnostic tests for brucellosis in cattle recently vaccinated against leptospirosis. J. *Vet. Diagn. Invest*., v.24, p.283-287, 2012.

FRANÇA, S.A.; MOL, J.P.S.; COSTA, E.A. *et al*. Indirect ELISA for diagnosis of *Brucella ovis* infection in rams. *Arq. Bras. Med. Vet. Zootec*., v.66, p.1695-1702, 2014.

GUSI, A.M.; BERTU, W.J.; MIGUEL, M.J. *et al*. Comparative performance of lateral flow immunochromatography, iELISA and Rose Bengal tests for the diagnosis of cattle, sheep, goat and swine brucellosis. *PLoS Negl. Trop. Dis*., v.13, p.1-10, 2019.

HERRERA, D.F.D.; SANTANA, Y.C.; SUI, O.C. *et al*. Development and evaluation of the performance of a rapid immunochromatographic test for the diagnosis of brucellosis. *Rev. Salud Anim.*, v.37, p.105-111, 2015.

HOBBS, E.C.; COLLING, A.; GURUNG, R.B. *et al*. The potential of diagnostic point-of-care tests (POCTs) for infectious and zoonotic animal diseases in developing countries: technical, regulatory and sociocultural considerations. *Transbound. Emerg. Dis.*, v.68, p.1835-1849, 2021.

KIM, D.; PARK, J.; KIM, S.J. *et al*. *Brucella* immunogenic BP26 forms a channel-like structure. *J. Mol. Biol.*, v.425, p.1119-1126, 2013.

KUMAR, S.; TUTEJA, U.; KUMAR, A. *et al*. Expression and purification of the 26 kDa periplasmic protein of *Brucella abortus*: a reagent for the diagnosis of bovine brucellosis. *Biotechnol. Appl. Biochem.*, v.49, p.213-218, 2008.

MANAT, Y.; SHUSTOV, A.V.; EVTEHOVA, E. *et al*. Expression, purification and immunochemical characterization of recombinant OMP28 protein of *Brucella* species. *Open Vet. J*., v.6, p.71-77, 2016.

MEIRELLES-BARTOLI, R.B.; MATHIAS, L.A. Estudo comparativo entre os testes adotados pelo PNCEBT para o diagnóstico sorológico da brucelose em bovinos. *Arq. Inst. Biol.*, v.77, p.11-17, 2010

POESTER, F.P.; NIELSEN, K.; SAMARTINO, L.E. *et al*. Diagnosis of brucellosis. *Open Vet. Sci. J*., v.4, p.46-60, 2010.

SILVA, T.M.A.; OLIVEIRA, R.G.; MOL, J.P.S. *et al*. Etiologic diagnosis of bovine infectious abortion by PCR. *Cienc. Rural*, v.39, p.2563-2570, 2009.

TIAN, M.; SONG, M.; YIN, Y. *et al*. Characterization of the main immunogenic proteins in Brucella infection for their application in diagnosis of brucellosis. *Comp. Immunol. Microbiol. Infect. Dis*., v.70, p.1-6, 2020.

TIWARI, A.K.; KUMAR, S.; PAL, V. *et al*. Evaluation of the recombinant 10-kilodalton immunodominant region of the BP26 protein of *Brucellaabortus* for specific diagnosis of bovine brucellosis. *Clin. Vaccine Immunol.*, v.18, p.1760-1764, 2011.