Letters in Animal Biology

Journal homepage: *www.liabjournal.com*

Comparison of Fluorescence Polarization Assay with Rose Bengal Plate Agglutination test and Competitive Enzyme-Linked Immunosorbent assay for bovine brucellosis in Tanzania

Godwin Minga,* Proscovia Kagaruki, Grace Banda, Zacharia Makondo, Henry Magwisha

Central Veterinary Laboratory (CVL), Tanzania Veterinary Laboratory Agency. Dar es Salaam, Tanzania

* Corresponding author:
   Godwin Minga

Email: godwinminga@yahoo.com

Reviewed by:
Richard Mwakapuja
   Tanzania Vaccine Institute, Tanzania
   Veterinary Laboratory Agency (TVLA),
   Kibaha, Tanzania

Jubeda Begum
   Department of Veterinary Microbiology, GB
   Pant University of Science and Technology,
   Pantnagar, Uttarakhand

Abstract

Brucella are Gram-negative, facultative, intracellular bacterial species with *B. abortus*, *B. melitensis, and B. suis* carrying the smooth-lipopolysaccharide antigen. Accurate diagnostic results of brucellosis are needed for its control and eradication, however, they are primarily based on the serological testing of brucellosis in animals. The Rose Bengal Plate Agglutination Test (RBPT) and competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) are the most commonly used tests for making such diagnosis. The use of Fluorescence Polarization Assay (FPA) in Tanzania is still in nascent stage. The purpose of this study was to compare RBPT, c-ELISA, and FPA in the diagnosis of bovine brucellosis. A total of 75 serum samples from cattle that were infected with *Brucella* in Kagera region were obtained. The FPA showed 90% (68 samples) prevalence, RBPT revealed 93% (70 samples) prevalence, and c-ELISA revealed 81% (61 samples) prevalence of brucellosis in the farm. The RBPT test has shown an inability to distinguish antibodies from cross-reacting organisms compared to the FPA test, while the c-ELISA was unable to pick a positive sample compared to the FPA test. FPA is very quick (5 min per sample), does not require specialized staff, and may be performed under field conditions. Therefore, FPA has a potential to overcome limitations in the detection of bovine brucellosis and can be used as a confirmatory test.

1. Introduction

Brucellosis in domestic animals is caused by Gram-negative bacteria which are facultative intracellular pathogens infecting humans as well. In *Brucella* genus eight species have recognized *viz. B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae, B. cetaceae, and B. pinnipediae* (Corbel et al. 1983; Foster et al. 2003; OIE 2008). *B. abortus*, *B. melitensis*, and *B. suis* are among the species that carry the smooth- Lipopolysaccharide (LPS) antigen (Cardoso et al. 2006). Brucellosis has important implications on veterinary and public health concerns. The serological testing of farm animals has been very instrumental in control and eradication of brucellosis (Nielsen et al. 2002; OIE 2011). The Rose Bengal Plate Agglutination test (RBPT), complement fixation test, and more recently Enzyme-Linked Immunosorbent Assay (ELISA) are commonly used in the diagnosis purpose. However, the history of Fluorescence Polarization Assay (FPA)

use in disease diagnosis is very recent and thus is not still considered as an established routine testing procedure in majority of the National Reference Laboratories for Brucellosis (OIE 2008; Nielsen et al. 2001). FPA, as the replacement of the CFT, is used to detect specific antibodies in serum against species of the genus *Brucella* which targets O Polysaccharide (OPS) extracted from *Brucella abortus,* which is the most specific antigenic part of LPS and conjugated with fluorophore. Their sensitivity and specificity is very high and can be used for screening and confirmation (Ellie Lab)

Accurate results of the RBPT and c-ELISA for brucellosis is a big challenge worldwide. This has a negative impact on the brucellosis control and eradication program (Godfroid et al. 2002; Munoz et al 2004). RBPT and c-ELISA continue to be routine tests for brucellosis as the screening and confirmatory tests, respectively. However, the major issue with RBPT and c-ELISA in diagnosis of brucellosis is their restricted specificity
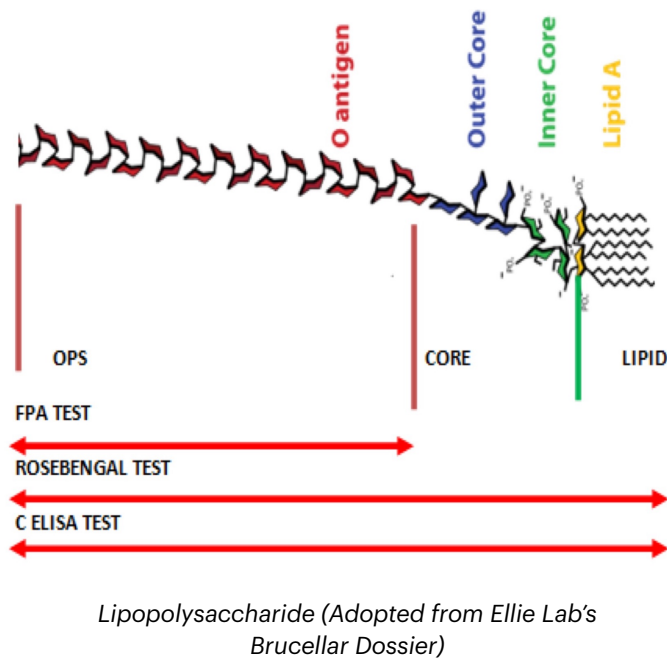
Lipopolysaccharide (Adopted from Ellie Lab's Brucellar Dossier)

in differentiating the *Brucella* LPS O-antigen side chain from other microbes such as *Yersinia enterocolitica* O:9 (Nielsen et al. 2004; Gwida et al. 2011).

In efforts to improve serological diagnosis, generally at least two tests are recommended to be used simultaneously to reduce the chances of false positive or false negative results (OIE 2008; Mc Given et al. 2006; Weiner et al. 2010). But some techniques, like c-ELISA, are expensive, time-consuming, and laborious and thus cannot be affordable in many developing countries like Tanzania. As a result of these facts FPA is gaining popularity in the diagnosis of brucellosis in animal herds. Because FPA is very quick (5 min per sample), does not require specialized staff, may be performed under field conditions, and data are obtained electronically (Gall et al. 2000; Lucero et al. 2003; OIE 2008; Mc Given et al. 2003; Minas et al. 2007). Despite all effort made to ensure accurate diagnosis results, comparison performance of the RBPT, c-ELISA, and FPA have not been carried out in Tanzania. FPA is recommended by the world organization for Animal Health (WOAH) for screening and confirmation of Brucella infection by *B. abortus* (Ellie Lab). Therefore, the aim of this study was to compare the performance of FPA, RBPT and c-ELISA in the diagnosis of brucellosis in cattle.

2. Materials and methods

Cross sectional study was undertaken at Kagera regional in Tanzania from December 2021 to February 2022 whereby 75 sera samples from cattle, originated from a highly infected farm with cases of abortion and stillbirth. The sera were obtained from Kagera Farm in 2021 and sent to the Central Veterinary Laboratory of Tanzania Veterinary Laboratory Agency in Dar es Salaam for further investigation. The sera

samples were stored at refrigeration temperature (2-8 °C) and half an hour before testing they were kept at room temperature.

RBPT was done according to official instruction protocols of Tanzania Veterinary Laboratory Agency SOP number 35. 30 ml of each antigen and serum sample were mixed on a clean glass slide using a stirring stick. It was followed by manual rotation of glass slide for 4 minutes to check for any degree of agglutination according to the protocol. If the agglutination occurred the test was declared positive. The c-ELISA test was performed by a commercial c-ELISA kit for Brucellosis following the instructions of the manufacturer (SVANOVIR, Sweden). The FPA test was conducted in borosilicate disposable glass (VWR, USA). Initially, 1 ml of a dilution buffer was placed into four 10x75 mm borosilicate tubes, and then 10 µl of negative control were added to three of them and positive control to the remaining one. Both control samples were obtained from in-house control. Samples were mixed, incubated at room temperature for 5 min, and an initial reading, referred to as the blank intensity reading, was taken using the FPA Reader Sentry 100 (Diachemix, USA-ellie Lab). Subsequently, 10 µl of a conjugate was added to each sample, and after mixing and incubation at room temperature for 3 min, a second reading was taken, referred to as a sample reading. Then the values for each sample are calculated by the reader automatically by subtracting the initial reading from second reading and expressed in millipolarization units (mP).

3. Results

All 75 samples were obtained from a highly infected farm. The FPA test has shown 90% prevalence of brucellosis in the farm, RBPT 93%, and c-ELISA 81% (Table 1). Taking all the tests together, the prevalence of brucellosis in Kagera farm was 88.4% with RBPT showing the most positive reactions (93%), the FPA showing positive in 90% animals, and c-ELISA showing the least positive reactions (81%).

Table 1 The results of tested samples by different serological tests

Test	Positive (%)	Negative (%)	Total (%)
RBPT	70 (93%)	5 (7%)	75 (100%)
c-ELISA	61 (81%)	14 (19%)	75 (100%)
FPA	68 (90%)	7 (10%)	75 (100%)

RBPT: Rose Bengal Plate Agglutination Test; c-ELISA: Competitive Enzyme-Linked Immunosorbent Assay; FPA: Fluorescence Polarization Assay

4. Discussion

This study shows that *Brucellar* infection on the Kagera cattle farm is relatively high. Overall prevalence was 88.4%. The RBPT test showed a high number of positive samples of *Brucella spp.,* similar to the study reported by Muma et al. (2009). On the other hand, this study showed that two sera

samples positive in RBPT were negative in FPA. Such discrepancies between the results of FPA and RBPT have been reported earlier as well (Weiner et al. 2010). Moreover, the RBPT has an inherent inability to distinguish antibodies from cross-reacting organisms present in the test sera, which would potentially result in false positive outcomes (Gall and Nelsen 2004). Another drawback of the RBPT is that only a good quality serum yields a desired result, whereas such interference in detection of serum antibodies in the FPA and c-ELISA is not observed by use of whole blood or hemolyzed serum (Nielsen et al. 2005).

For FPA, the study showed that the test detected a high number of *Brucella* reactors (68) whereas, c-ELISA detected 61. This is similar to the study by Gwida et al. (2011), which reported that FPA obtained the highest number of positive samples compared to c-ELISA. The FPA has the ability, in some cases, to distinguish antibodies from cross-reacting organisms (e.g. *Y. enterocolitica* O:9) from antibodies against *Brucella spp.* and is slightly superior to the c-ELISA (Gwida et al. 2011). The diagnostic value of RBPT and c-ELISA for brucellosis is restricted by their low specificity in differentiating the *Brucella* LPS O-antigen side chain from other microbes such as *Yersinia enterocolitica* O:9 (Nielsen et al. 2004; Gwida et al. 2011). RBPT is the most economical and widely used laboratory test, but the interpretation of its results is largely subjective. In efforts to improve serological diagnosis, generally at least two tests are recommended to be used simultaneously to reduce the chances of false positive or false negative outcomes (OIE 2008; Weiner et al. 2010).

5. Conclusions

Compared to FPA, the interpretation of RBPT results is subjective with greater chances of false positive results. Furthermore, FPA will be more suitable for the identification of *Brucella* than c-ELISA; moreover*,* c-ELISA requires expert personnel for operation and sophisticated equipment to achieve the results which is not generally possible under field conditions. Therefore, under such conditions FPA can be used as a diagnostic test of choice.

Recommendation

Quick and accurate diagnosis results of brucellosis is very important for effective Brucella control and eradication programs. Being a rapid test, objective in results, and economical the FPA should be used for routine bovine brucellosis examination. However, to ascertain the reproducibility of FPA further detailed studies are required.

Declarations

Funding: Not available

Conflict of interest: The authors declare no conflict of interest arising out of the work reported in this paper

Ethical approval: Not applicable

References

Cardoso PG, Macedo CG, Azevedo V, Oliveira CS. (2006). Review, *Brucella spp* non canonical LPS: structure, biosynthesis and interaction with host immune system; Microbial Cell Factories 5: 13. https://doi.org/10.1186/1475-2859-5-13

Corbel MJ, Gill KPW, Thomas EL. (1983). Methods for the identification of *Brucella*. Central Veterinary Laboratory, New Haw, Wey bridge, UK. Pp. 1–63.

Foster G, MacMillan AP, Godfroid J, Howie F, Ross HM, Cloeckaert A, Reid RJ, Brew S, Patterson IAP. (2002). A review of *Brucella sp.* infection of sea mammals with particular emphasis on isolates from Scotland. Veterinary Microbiology 36: 563-580.

Gall D, Nielsen K, Forbes L, Davis D, Elzer P, Olsen S, Balsevicius S, Kelly L, Smith P, Tan S, Joly D. (2000). Validation of the fluorescence polarization assay and comparison to other serological assays for the detection of serum antibodies to *Brucella abortus* in bison. Journal of Wildlife Diseases 36: 469-476.

Gall D, Nielsen K. (2004). Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. Revue Scientifique et Technique – OIE 23(3): 989-1002.

Godfroid J, Saegerman C, Wellemansa V. (2002). How to substantiate eradication of Bovine brucellosis when a specific serological reactions occur in the course of brucellosis testing. Veterinary Microbiology 90: 461-477.

Gwida MM, El-Gohary AH, Melzer F, Tomaso H, Rosler U, Wernery U, Wernery R, Elschner CM, Khan I, Eickhoff M, Schoner D, Neubauer H. (2011). Comparison of diagnostic tests for the detection of *Brucella spp.* in camel sera. BMC Research Notes 4: 525. https://doi.org/10.1186/1756-0500-4-525

Lucero NE, Escobar GI, Ayala SM, Silva Paulo P, Nielsen K. (2003). Fluorescence polarization assay for diagnosis of Human brucellosis. Journal of Medical Microbiology 52: 883-887.

Mc Given JA, Stack JA, Perrett LL, Tucker JD, Brew SD, Stubberfield E, MacMillan AP. (2006). Harmonisation of European tests for serological diagnosis of infection in bovines. Revue Scientifique et Technique – OIE 25(3): 1039-1053.

Mc Given JA, Tucker JD, Perrett LL, Stack JA, Brew SD, MacMillan AP. (2003). Validation of FPA and c-ELISA for the detection of antibodies to *Brucella abortus* in cattle sera and comparison to SAT, CFT, and iELISA. Journal of Immunological Methods 278: 171-178.

Minas A, Stournara A, Minas M, Stack J, Petridou E, Christodoulopoulos G, Krikelis V. (2007). Validation of a fluorescence polarization assay (FPA) performed in microplates and comparison with other tests used for diagnosing *B. melitensis* infection in sheep and goats. Journal of Immunological Methods 320, 94-103.

Muma JB, Lund A, Nielsen K, Matope G, Munyeme M, Mwacalimba K, Skjerve E. (2009). Effectiveness of Rose Bengal test and

fluorescence polarization assay in the diagnosis of *Brucella spp.* Infections in free range cattle reared in endemic areas in Zambia. Tropical Animal Health and Production 41: 723-729.

Munoz P, Marin CM, Monreal D. (2004). Efficacy of several serological tests and antigens for diagnosis of Cattle brucellosis in the presence of false positive serological results due to *Yersinia enterocolitica O:9*. Clinical and Diagnostic Laboratory Immunology 12: 141-151.

Nielsen K, Gall D, Bermudek R, Renteria T, Moreno F, Corral A, Monroy O, Monge F, Smith P, Widdison J, Mardrueno M, Calderon N, Guerrero N, Tinoco R, Osuna J, Kelly W. (2002). Field trial of the brucellosis fluorescence polarization assay. Journal of Immunoassay and Immunochemistry 23: 307-316.

Nielsen K, Gall D, Smith P, Kelly W, Yeo J, Kenny K, Heneghan T, McNamara S, Maher P, O'Connor J, Walsh B, Carroll J, Rojas X, Rojas F, Perez B, Wolff O, Bufoni L, Salustio E, Gregoret R, Samartino L, Dajer A, Luna-Martinez E. (2001). Fluorescence polarization assay for the diagnosis of Bovine brucellosis: adaptation to field use. Veterinary Microbiology 80: 163-170.

Nielsen K, Smith P, Widdison J, Gall D, Kelly L, Kelly W, Nicoletti P. (2004). Serological relationship between cattle exposed to *Brucella abortus, Yersinia enterocolitica O:9* and *Escherichia coli O:157:H7*. Veterinary Microbiology 100: 25-30.

Nielsen K, Smith P, Yu W, Nicoletti P, Elzer P, Robles C, Bermudez R, Renteria T, Moreno FS, Ruiz A, Massengill C, Muenks Q, Jurgersen G, Tollersrud T, Samartino L, Conde S, Forbes L, Gall D, Perez B, Rojas X, Minas A. (2005). Towards single screening tests for brucellosis. Revue Scientifique et Technique - OIE 24(3): 1027-1037.

OIE. (2008). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, Paris, France.

OIE. (2011). Bovine brucellosis. In: Manual of diagnostic tests and vaccines for terrestrial animals. World Organization for Animal Health, Paris, France. Pp. 1-35.

Weiner M, Iwaniak W, Zlotnicka J, Szulowski K. (2010). Diagnosis of Bovine brucellosis using traditional serological techniques and Fluoresence polarization assay. Bulletin of the Veterinary Institute in Pulawy 54: 485-488.

Citation