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Assessment of Fluorescence Polarization Titres as a Prediction Indicator of Which *Brucella* Spp. Circulate in a Domestic Ruminant Animal Populations

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Abstract

Brucellosis is an important zoonotic bacterial disease and has been an emerging disease since the discovery of Brucella melitensis by David Bruce in 1887. Eradication programmes date back to 1905, based on vaccination, test and slaughter, and/or different combinations of these potential strategies. It is widely accepted that vaccination and serological tests have played a significant role in the successful control of brucellosis. Serological tests such as, Rose Bengal test, Rivanol test, complement fixation test, enzyme link immunoassay tests were developed earlier, while Brucella Fluorescence Polarization Assay (FPA) was developed almost two decades ago. This uses a subunit of Lipopolysaccharide, O-chain polysaccharide, the most specific and sensitive antigenic determinant of Brucella. Based on its high specificity and sensitivity, the FPA is used for various purposes: screening, confirmation of diagnosis of Brucellosis and discrimination of vaccination and infected animals. There was a significant difference between antibody titres with the two FPA kits used. The highest antibody titre was produced when the type of FPA kit matched with the Brucella spp. Hypothetically, FPA may be used to predict species of Brucella circulating in brucella affected herds.

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INTRODUCTION

Brucellosis is a bacterial zoonotic disease that affects domestic and wild animals, including humans [4, 10, 11, 12]. The disease is caused by various bacteria in the family *Brucella*, which tend to infect specific animal species where *Brucella* spp are well adapted, however most species of *Brucella* can also infect other animal species. Today, the *Brucella* genus has at least 12 species, some with high zoonotic potential. Brucellosis in cattle (*B. abortus*) in sheep and goats (*B. melitensis*) and in swine (*B. suis*) are OIE listed diseases [4, 10]]. A range of domestic animals such cattle, swine, sheep and goats, camels, equines, and dogs are susceptible to infection. It may also infect other ruminants, some marine mammals and humans [11, 12]. The disease in animals is mainly characterized by abortion in pregnant animals, orchitis and epididymitis in males and joint hygroma in both males and females. Abortion occurs only in the first pregnancy after infection, usually in the second period of pregnancy. Infected animals shed the bacteria mostly at abortion or at calving/lambing; however, infected animals also shed the bacteria with other exertions, secretion and fluids. While animals typically recover and will be able to have live offspring following the initial abortion, they may continue to shed the bacteria [4, 10, 11, 12].

The zoonotic and economic importance of brucellosis justifies the implementation of national strategies for its control [11, 12]. One of most important tools for the successful control of brucellosis is establishing a functional diagnostic system and the availability of valid diagnostic tests with high reliability [4]. The diagnosis of brucellosis in cattle is based on bacteriological, immunological and molecular tests. Isolation of *Brucella* spp., remains the only gold standard method, however its sensitivity is not 100%, the method is complex, it requires well equipped laboratory facilities and well trained staff. Microbiological methods are not suitable for use on a large scale; *Brucella* can be isolated from over 80 or even 90% of suspected individuals [4].

Immunological methods can detect antibodies induced by *Brucella* spp. but are not able to detect the presence of live bacteria or individual shedders of *Brucella* spp [1, 2]. It is known that animals that show positive serological results do not always shed the bacteria. In addition, approximately 35% of serological positive animals are resistant to infection and although they are resistant they may show positive results in serological tests [4].

Vibrio cholerae O1, *Escherichia coli* O: 157, *Escherichia hermannii* and *Stenotrophomonas maltophila*, *Salmonella* group, and *Yersinia enterocolitica* O:9 can be responsible for false-positive serological reactions in the diagnosis of bovine brucellosis []10, 11, 12]. Molecular methods are suitable for detecting the presence of DNA of *Brucella* spp., but they are expensive, require advanced laboratory facilities and are not able to discriminate genetic material from live or dead bacteria [4, 10]. The BRUCELLA FPA is a diagnostic test using fluorescence polarization assay (FPA) technology designed to determine the presence of specific antibodies in serum, plasma or milk samples, against species of the genus *Brucella* that produce smooth colonies (*B. melitensis, B. abortus* and *B. suis* [2, 3]. The presence of antibodies is indicative of prior infection with *Brucella*. The diagnostic test uses an O-polysaccharide (OPS) extracted from *Brucella abortus* and *B. meitensis* bacteria and conjugated with a fluorophore. OPS is the most specific antigen and is a component of the *Brucella* lipopolysaccharide (LPS). The immune response to OPS is correlated with active infection and bacterial load [2, 4, 8, 9, 13]. Animals that clear infection, as in the case of vaccination, soon become negative on the FPA [2, 4, 14, 15]. Fluorescence polarization is a homogenous assay. The reaction is read directly in the tube, micro-plate or stripwells without washing steps or secondary reagents [2, 9]. This allows for a simple, rapid and accurate test. The assay time is only a few minutes and can be done in the field or laboratory [15]. The test can be manual or fully automated, in which case large numbers of samples can be processed in a short time [2, 9, 15]. In all species, OPS is an immuno-dominant antigen. Therefore, the Brucella FPA is successfully used and validated for testing cattle, sheep, goats, swine, cervids, bison, buffalo, camels and other species, including humans. Because Brucella FPA detects only antibodies against OPS, diagnostic sensitivity and diagnostic specificity of the assay are very high.

Therefore, the test can be used as a screening or confirmatory test, or as an aid in distinguishing vaccinated from infected herds [2, 4, 6, 7, 9, 10, 15]. The test is validated by many veterinary authorities and is the main confirmatory test for cattle in most of the countries of the Americas, including the United States, Canada, Argentina, Brazil, Uruguay, Colombia and other nations [4, 10]. The FPA is approved in the European Union (EU) for testing cattle for trade between member states. In China, it is approved for testing for export and import. It is also used by several large dairies for brucellosis control and eradication. Many other countries are switching over to use the Brucella FPA as either a confirmatory or a screening assay [10].

Comparing the serological tests there are obvious differences between them and there is a need for rational and strategic use of them, depending on aims, status and prevalence of disease, test viability, costs and stage of the control programme. Every test used has its specific purpose and parameters, shown very briefly as follows: The different serological tests have different features and measures. Buffered Acidified Plate Antigen (BAPA) is a screening test, it is rapid, automated and may be used to classify cattle as negative. The test records results as either negative or positive by measuring IgG_1, IgG_2 and IgM. It is excellent for screening purposes with a good sensitivity and likelihood-ratio LR (+) = ~37. BRT (Brucellosis Ring Test) is an excellent screening test for dairies, it is a cheap test with good range of sensitivity. Fluorescence Polarization assay (FPA) is a confirmatory test, fast, cheap and easy to perform even in field conditions by measuring $IgG_{1\&2}$. It may be used in milk, blood, sera and plasma. In comparison to other serological tests available it is a very good test with a likelihood-ratio LR (+) = ~97. cELISA is used for screening and confirmatory purposes by measuring $IgG_{1\&2}$ which could be used on both sera and milk samples. It has good sensitivity and specificity

Brucellosis is most important zoonotic infectious disease and it is still endemic in Albania. A national control programme of *B. melitensis* has been in place in Albania since 2012 based on mass vaccination of small ruminants, and since 2016 there has been an active surveillance programme in dairy cattle [4]. Most recent data indicate that the majority of the national cattle herd is circulating *B. abortus*. However, as co-grazing of small ruminants and cattle is a

common practice, there is a high potential risk for infection of cattle by *Brucella melitensis*. Isolation and identification of *Brucella* spp. is important for any appropriate control programme, but because of risks and its complexities, isolation is not a routine bacteriological procedure. It is known and accepted that FPA has been developed to distinguish infected from vaccinated animals [4, 9, 10, 15].
The aim of this study was to assess the usefulness of FPA for judging which *Brucella* spp. is circulating in the national cattle population of Albania.

MATERIALS AND METHOD

Five sera blood samples of culture-positive cattle for *Brucella abortus* and 152 sera blood samples from 76 vaccinated goats with *Brucella melitensis* Rev. 1 vaccine strain were used. Sera sample were tested in parallel with an FPA *Brucella abortus* kit and an FPA *Brucella melitensis* kit. (Ellie Headquarters Milwaukee, U.S.A United States). Sera were diluted in distilled water at a 1:25 and 1:10 ratios respectively. The test procedure was performed in 10x75 mm borosilicate glass test tubes. For single tube FPA instrument glass tubes; 20µl of samples and controls in 1 ml diluted samples diluent were pipetted. Negative controls were run in triplicate, while positive control and samples as single tests. After mixing, the samples were incubated (3-30 minutes) at room temperature and a first (blank) reading was obtained using Sentry® Software 2.3.26.exe. The tracer was added (10µl) to all samples and controls, after 2-5 minutes a second reading was taken, and millipolarisation (mP) units were recorded. The results of the FPA tests were expressed as delta mP (ΔmP) values of the samples and were calculated as the difference between mP value of the samples and the mean of the negative control mP values. The student t-Test Paired Two Sample for Means tool was used to analyze the data.

RESULTS AND DISCUSSION

In total, there were tested five bovine sera samples and 150 sera goat samples. The criterion used for determining the status of animal-tested based on FPA was the titre expressed in ΔmP. The animals

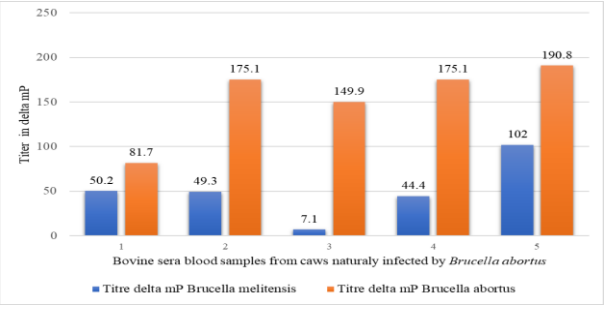
that produced a titre under 10 ΔmP were considered free of infection, while animals that showed a titre of between 10 – 20 ΔmP were considered doubtful (suspicious or suspect), and animals that produced a titre higher than 20 ΔmP were considered positive.

The FPA results are show in Table 1, Table 2 and Figures 1 – 3.

Table 1 – FPA antibody titre of bovine sera samples using the FPA *B. abortus* and FPA *B. melitensis* Kits, respectively. The sera samples belong to five culture positive caws.

B. abortus culture Positive samples	Titre ΔmP *Brucella melitensis*	Titre ΔmP *Brucella abortus*	Titre Difference
1	50.2	81.7	**31.5**
2	49.3	175.1	**125.8**
3	7.1	149.9	**142.8**
4	44.4	175.1	**130.7**
5	102.0	190.8	**88.8**

Figure 1 – FPA antibody titre of bovine sera samples using the FPA *B. abortus* and FPA *B. melitensis* Kits, respectively. The sera samples belong to five culture positive caws. There is an apparent titer difference between samples tested by different *Brucella melitensis* and *Brucella abortus*.



The results show that mean titre of bovine sera tested with FPA *Brucella melitensis* kit was 50.6 ΔmP compared to the mean titre of 154. 5 ΔmP (Table 1) obtained when samples were tested with the FPA *Brucella abortus* kit. There was a significant difference between samples tested by different *Brucella melitensis* and *Brucella abortus* ($P<0.05$) t_{stat} ($t = 5.1$) which was greater than t_{tab} ($t = 2.8$) between the groups.

We failed to have access to sera from sheep or goats naturally infected with *B.melitensis.* In those circumstances, sera blood samples from goats vaccinated with *B. melitensis* Rev 1 strain were used. The sera from 76 vaccinated goats bled after 21 and 45 days were tested in parallel with both FPA kits.

Table 2 – FPA antibody titre of goat sera samples vaccinated with *B. melitensis* Rev 1 using the FPA *B. abortus* and FPA *B. melitensis* kits, respectively. There was an apparent higher titre on samples tested with FPA *Brucella melitensis* kit compared with titers produced from the FPA *Brucella abortus* kit.

Parameters	*Brucella abortus* tracer	*Brucella melitensis* tracer ΔmP	*Brucella abortus* tracer - ΔmP	*Brucella melitensis* tracer ΔmP
	Titre expressed as ΔmP Day 21 PV		Titre expressed as ΔmP Day 45 PV	
Mean	53.4	68.9	46.9	90.6
Median	41.5	56.5	37	94
Standard deviation	43.7	52.7	36.98	46.9
Maximum	172	181	134	170

Results indicate that 21 days after vaccination there was a significant difference between samples tested with the two different FPA *Brucella* kits ($P < 0.05$, $t = 3.9$). The antibody titre (mean titre 69.0 ΔmP) from FPA *B. melitensis* compare to FPA *B. abortus* (mean titer 52.4 ΔmP) was higher. The titre difference was significantly much higher ($P< 0.005$; $t = 9.7$) 45 days post vaccination 91.3 ΔmP FPA *B. melitensis* compare 45.9 ΔmP FPA *B. abortus.*

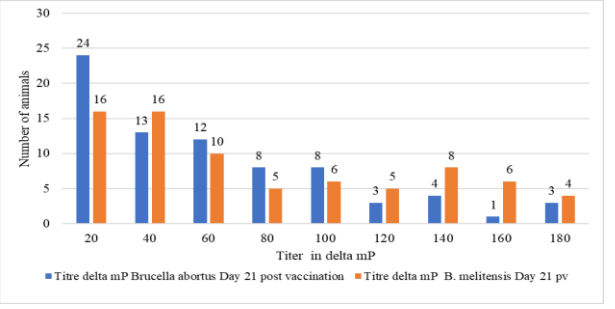


Figure 2 – FPA antibody Titre of goat sera samples vaccinated with *B. melitensis* Rev 1 using the FPA *B. abortus* (marked in blue colour) and FPA *B. melitensis* kits (marked in red colour), respectively.

The animals that were bled 21 days post vaccination, gave sera blood samples that were preserved at -20^{0}C until testing. The titre expressed in ΔmP ranged from 0 to 181ΔmP. The results were classified into nine groups (0-20 ΔmP; 21-40 ΔmP; 41-60 ΔmP; 61-80 ΔmP; 81-100 ΔmP; 101-120 ΔmP; 121-140 ΔmP; 141-160 ΔmP; and

161-181 ΔmP) and tabulated in Figure 2. There is a significant higher titre with samples tested with FPA *Brucella melitensis* kit compared to titres produced with the FPA *Brucella abortus* kit. A total of 23 out of 76 sera goat samples (30%) tested with FPA *B. melitensis* produce titres >100 ΔmP, compared with 11 sera goat samples (14.5%) tested with FPA *B. abortus.*

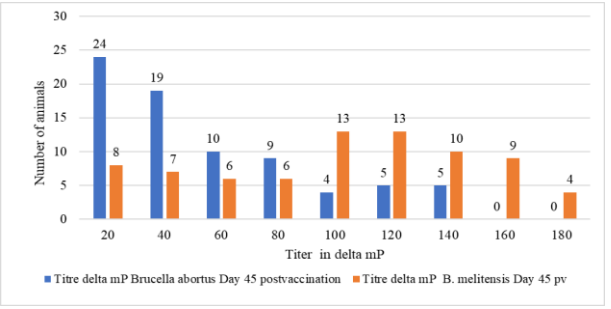


Figure 3 – FPA antibody Titer of goat sera samples vaccinated with *B. melitensis* Rev 1 by using the FPA *B. abortus* and FPA *B. melitensis* kits, respectively.

The animals that were bled 45 days post vaccination gave sera blood samples that were preserved at -20^{0}C until testing. The titre expressed in ΔmP ranged from 0 to 180ΔmP. The results were classified into nine groups (0-20 ΔmP; 21-40 ΔmP; 41-60 ΔmP; 61-80 ΔmP; 81-100 ΔmP; 101-120 ΔmP; 121-140 ΔmP; 141-160 ΔmP; and 161-180 ΔmP) and tabulated in Figure 3. There was an apparent higher titre on samples tested with the FPA *Brucella melitensis* kit compared with the titre produced from the FPA *Brucella abortus* kit. There were no sera samples that generate titres higher 140 ΔmP tested with FPA *B. abortus.* In addition, the majority of the sera samples (63 sera samples or 83%) produce lower titres (<80ΔmP) when they were tested by FPA *B. abortus,* while most of samples (49 sera goat samples or 64.5%) tested by FPA *B. melitensis* kit produced titres > 80 ΔmP.

This study has a clear limitation: the number of tested samples from culture-positive cattle was relatively low. There was also a lack of positive sera from infected goats and sheep confirmed by isolation of *Brucella melitensis*.

Further work is needed to investigate the differences in the large numbers of *Brucella* spp in culture-positive cattle, sheep and goats.

CONCLUSION

There was a significantly higher titre difference from *B. abortus* infected cattle tested with FPA *B. abortus* kit compare with titres from the FPA *B. melitensis* kit. A similar pattern was found when sera blood samples from vaccinated goats were tested by the same method but using the different kits. The ΔmP titres of vaccinated goats were significantly higher when tested with FPA *B. melitensis* compared with titers generated when samples were tested with FPA *Bruvcella abortus.* These data indicate that higher antibody titres to *B. abortus* are obtained when bovine sera blood samples are tested with FPA *B. abortus,* in this study it was three times higher. The FPA antibody titres for *B. melitensis* Rev 1 vaccine strain were 24.1% higher when tested with FPA *B. melitensis* compare with antibody titers generated when tested with FPA *B. abortus* 21 days post vaccination, while the difference was double at 45 days post vaccination. There is therefore evidence that FPA may indicate which *Brucella* spp. are circulating in a serologically positive herd.

REFERENCES

1. Ducrotoy, Marie J., Munoz, Pilar M., Conde Alvarez, Raquel, Blasco, Jose M., Moriy on, Ignacio (2018). A systematic review of current ´ immunological tests for the diagnosis of cattle brucellosis. Preventive Veterinary Medicine. https://doi.org/10.1016/j.prevetmed
2. Gall D, Nielsen K (2004). Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. Rev Sci Tech. 23(3):989-1002.
3. Klaus Nilsen (2002). Diagnosis of brucellosis by serology. Vet. microbiology 90, 447 – 459.
4. Koleci Xh and Keçi Ruzhdi (2017). Brucellosis In: Infectious Disease and Preventive veterinary Medicine, 435 -470.

5. Markey Bryan, Leonard Finola, Archambault Marie, Cullinane Ann, Maguire Dores (2013). *Brucella* species In: Clinical Veterinary Microbiology second edition. Chapter 23
6. McGiven JA, Stack JA, Perrett LL, Tucker JD, Brew SD, Stubberfield E, MacMillan AP (2006). Harmonization of European tests for serological diagnosis of Brucella infection in bovines. Rev Sci Tech..
7. Minas, A. Stournara, M. Minas, J. Stack, E. Petridou, G. Christod oulopoulos, V. Krikel (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. J. Immu. Meths., 320, 94-103.
8. Nicola A.M., Elena S., Alonso B., Esteves M.J., (2010). Evaluation of the Fluorescence Polarization Assay (FPA) for diagnosis of *Brucella melitensis* infection of Goats in Argentina. Prilozi, 31, 133-143
9. Nielsen K, Gall DJ (2001) Fluorescence polarization assay for the diagnosis of brucellosis: a review. Immunoassay Immunochemistry. 22(3):183-201.
10. OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 3.1.4 (2018).
    Brucellosis (*Brucella abortus*, *B. melitensis* and *B.suis*), online version.
11. Otto Radostits Clive Gay Kenneth Hinchcliff Peter Constable (2007). Brucellosis In: Veterinary Medicine 10th Edition. A textbook of the diseases of cattle, horses, sheep, pigs and goats
12. Quinn P.J., Markey B.K., Donnelly W.J., Leonard F.C., Fanning S. and Maguire D. *Brucella* species. In: Veterinary Microbiology and Microbial Disease, 2nd Edition; 2011: 334-341.
13. Schumaker B.A., Corso B.A., Rhyan J.C., Philo L.M., Salman M.D., Gardner I.A., (2010). Evaluation of the fluorescence polarization assay for the detection o**f** *Brucella abortus* antibodies in bison in a natural setting. Comp. Immunol. Microbiol. Infect. Dis., 33, pp. e119-e125, 10.1016/j.cimid.2010.07.001
14. Trangadia B.J., Nagmani K., Rana S.K., Srinivasan V.A., Montagnaro S. (2012). Evaluation of fluorescence

polarization assay for the diagnosis of brucellosis in cattle and buffaloes in India. Indian. J. Anim. Sci., 82, 561-564

15. USDA APHIS report (2004). Fluorescence polarization assay TEST Field Trials and testing results for validation of the FP Test in cattle, bison, and swine https://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/downloads/fpa-val-rpt.pdf

Supplementary table

Nr	*Brucella abortus* tracer	*Brucella melitensis* tracer ΔmP	*Brucella abortus* tracer - ΔmP	*Brucella melitensis* tracer ΔmP
	Titre expressed as ΔmP Day 21 PV		Titre expressed as ΔmP Day 45 PV	
1	128	58	117	35
2	156	179	134	170
3	12	10	15	129
4	74	156	68	69
5	7	17	17	97
6	30	9	22	11
7	41	25	55	50
8	19	38	8	0
9	16	51	41	110
10	49	80	19	36
11	26	8	40	104
12	42	76	28	102
13	62	23	102	153
14	11	49	10	100
15	36	26	45	91
16	47	143	44	148
17	13	3	13	14
18	19	43	19	35
19	15	28	11	13
20	49	96	46	123
21	88	128	75	106
22	90	150	73	136
23	0	0	0	0
24	164	181	105	160
25	69	144	53	113
26	19	59	69	108
27	13	12	25	82
28	74	107	27	72
29	18	111	39	167
30	170	177	125	167
31	17	32	15	61
32	44	90	17	40
33	34	122	16	53
34	16	13	10	87
35	107	144	97	152
36	16	40	25	111
37	87	80	128	103
38	21	7	61	30
39	3	1	5	50
40	87	145	66	146
41	4	80	5	158
42	19	17	37	82
43	8	2	6	88

44	53	92	59	124
45	22	56	25	90
46	66	88	43	67
47	87	111	67	117
48	0	0	0	0
49	125	131	86	101
50	15	16	16	20
51	15	127	13	141
52	60	23	29	81
53	50	35	20	66
54	66	55	51	101
55	38	31	14	58
56	90	63	29	47
57	64	128	67	143
58	55	28	87	26
59	15	38	39	112
60	49	105	65	88
61	24	42	19	82
62	11	8	28	47
63	39	57	20	87
64	126	59	100	86
65	95	37	33	18
66	33	9	11	123
67	36	30	31	111
68	111	89	115	144
69	34	30	25	37
70	103	95	126	131
71	172	137	118	133
72	45	27	37	68
73	93	121	57	140
74	35	117	30	128
75	140	166	132	170
76	75	124	37	137