Conjugation Of Lipopolysaccharide Brucella Antigen With Fluorescein Isothiocyanate For Detection Of Brucellosis

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ABSTRACT

Serological tests were developed for the detection of antibodies against Brucella species. In the present study, lipopolysaccharide of Brucella abortus S99 was conjugated with fluorescein isothiocyanate (FITC) and used for detection of Brucellosis in comparison with other serological tests as Rose bengal test (RBT), competitive enzyme linked immunosorbent assay (C-ELISA) and indirect enzyme linked immunosorbent assay (I-ELISA). The sensitivity of fluorescent antibody technique (FAT) and c-ELISA was 100% and 90% respectively, while that of I-ELISA and RBT was 75% and 81% respectively in cattle sera. The relative specificity of FAT was 100%, 96% and 92.8% in cattle, sheep and goat sera, respectively. The FITC is the test of choice for diagnosis of Brucellosis because it has the best sensitivity and specificity values comparable to the C-ELISA, I-ELISA and RBT.

INTRODUCTION

Brucellosis is a major zoonotic disease widely distributed in humans, domestic and wild animals especially in developing countries. Among different species of the Brucella genus abortus and Brucella melitensis are the most pathogenic and virulent, not only in cattle, sheep and goats but also for other animals species. The occurrence of the disease in humans is largely dependent on the animal reservoir, with the highest rate of human infection in areas whose rates of brucellosis in sheep and goats are high *(1)*. Therefore, serological tests were developed for the detection of antibodies to Brucella abortus in cattle.

Primary birding assay for the detection of antibodies to Brucella species in cattle were developed to improve the test sensitivity and specificity over those of the traditional tests such as Rose Bengal test (RBT) and the tube agglutination test (TAT). The indirect enzyme immunoassay (I-ELISA) is highly sensitive. However, the I-ELISA can not differentiate vaccinal antibodies or antibody elicited by cross-reacting organisms from antibodies resulting from field infection with Brucella species. The competitive enzyme immunoassay (C-ELISA) and the fluorescent antibody technique (FAT) can often distinguish antibodies due to vaccination with Brucella abortus strain 19 from antibodies elicited by exposure to other pathogenic strains *(2)*.

The FAT, unlike, the ELISA, could be adapted for serological testing in the field with a subsequent reduction in submission costs, turn around time and animal handling.

In this study, the sensitivity and specificity values of the FAT were compared with other tests for presumptive diagnosis of brucellosis in farm animals.

MATERIAL AND METHODS

1. Animals and Experimental Schedule

a. Guinea pigs

Two groups of Guinea pigs weighing 300-350 grams body weight. The 1st groups (50 animals) vaccinated S/C with 1/15 of bovine dose reduced dose (9 x 10^8 CFU) for each animal. Brucella abortus strain 19 vaccine,.

The 2nd group (50 animals) was vaccinated S/C with 1/15 of bovine dose Brucella abortus strain 19 high dose (6 x 10^9 CFU) for each animal.

Serum samples were collected four months post vaccination and evaluated by different serological tests.

Guinea pigs were sacrificed 4 months post infection. The spleens of each group were

treated separately. Spleens were collected and weighed homogenized in 1 ml PBS, serially diluted and 200 µl aliquots of the dilutions were plated onto TSA (Tryptic Soya agar) and incubated for 4-8 days at 37°C. Recovered bacteria were enumerated to evaluate persistence of each individual organisms *(3)*.

b. Farm animals

Hundred animals suspected to be infected with Brucellosis, 16 cattle, 54 sheep and 30 goats. The collected samples (milk, semen and vaginal swabs) were cultivated in serum trypticase soya agar medium for isolation of Brucella species *(4)*. The positive cases of Brucella were tested serologically (RBT, I-ELISA, ELISA and FAT).

2. Preparation of FITC with LPS

a. Lipopolysaccharide of Brucella abortus 99 (LPS) antigen was extracted *(5)*.

b. Conjugation of LPS with fluorescein isothiocyanate *(6,7)*.

3. Serological Tests

1. RBT was performed as described by the **Office International des Epizootics Manual of Standard for Diagnostic Tests and Vaccines** *(8)*.
2. I-ELISA was done as described *(9)*. The inhibiting ELISA uses smooth lipopolysaccharide (SLPS) from Brucella abortus strain 1119.3 as the antigen adsorbed on to polystyrene microplate (NUNC).
3. C-ELISA was performed *(2)*.

Competitive ELISA uses also SLPS antigen adsorbed onto polystyrene microplate.

4. Data handling and analysis

The sensitivity and specificity of different serological tests were determined using Bayesian approach test *(10)*.

RESULTS AND DISCUSSION

The serological tests were developed for detection of antibodies for B. abortus in cattle and had been evaluated for the detection of antibody to Brucella suis in pigs. The sensitivity and the specificity in culture either positive or negative varied widely *(11)*.

The RBT was developed for the detection of antibody against Brucella species in bovine and porcine sera *(12)* not in plasma. Acidified antigen with pH 3.65 to 3.66 reduces the final pH of plasma and antigen mixture to approximately pH 4.0 that causes the fibrinogen in the plasma to form, fibrin. The fibrin may interfere with the agglutination sometimes or could be falsely interpreted as agglutination by inexperienced personal. This reduces the usefulness of RBT and CFT, but not I-ELISA, C- ELISA and FAT. The latter can be distinguished cross reacting antibodies from antibody to Brucella species reducing the number of false positive reactions in brucellosis tests in cattle. In this study, the

sensitivity and specificity of FAT comparing with the other serological tests were determined.

The vaccinated guinea pigs were examined serologically, FAT gave the highest specificity (84%) followed by C-ELISA (50%) and the lowest estimate was RBT (10%) as shown in Table 1 size cattle, 54 sheep and 30 goats were examined serologically for Brucellosis. Also, isolation of Brucella organisms from milk and vaginal swabs were occurred. FAT gave the highest specificity and sensitivity in comparison with the serological tests as shown in Table 2.

These results are similar to previous study with *(13)* which indicated that the sensitivity of FAT and C-ELISA were 100 % and 90% in cattle, while in sheep 96.7 % and 85.7% and in goats were 94.4 and 77.2, respectively.

The specificity of FAT and C-ELISA in cattle was 100 % and 87.8%, while in sheep 96.7% and 85.7% and in goats were 94.4 % and 77.2 %, respectively. The specificity of FAT and C-ELISA in cattle were 100 % and 87.8 %, in sheep 96% and in goats were 92.8 % and 72.2 %, respectively.

Both sensitivity and specificity in FAT and c-ELISA were higher than those of RBT and I-ELISA as shown in Table 2.

Table 1. Comparison of relative specificity for detection of antibody to brucellosis four months post guinea pig vaccination

Serological tests	Guinea pigs vaccinated with reduced dose ($9x10^8$ CFU)			Guinea pigs vaccinated with high dose ($9x10^8$ CFU)		
	Mean splenic colonies/gram					
	-ve	+ve	Specificity	-ve	+ve	Specificity
RBT	3	47	6%	5	45	10%
I-ELISA	5	45	10%	8	42	16%
C-ELISA	20	30	40%	25	25	50%
FAT	35	15	70%	42	8	84%

However, the relative specificity of C-ELISA and I-ELISA were higher than that of RBT, in cattle 87.8%, 87.8% and 77.7%, in sheep was 82.7%, 82.7% and 70%, while in goats were 72.2%, 81.2% and 65%, respectively. The improvement in the relative specificity was at least partly due to addition of EDTA in the dilution of sera reducing the non-specific protein interaction. FAT and C-ELISA can distinguish antibodies to Yersinia enterocolitiae O:9 from antibody to Brucella species. RBT does not distinguish between antibodies produced some other organisms and antibodies by Brucella species *(9)*.

A comparison of the specificity of RBT, I-ELISA, C-ELISA and FAT for serum samples of vaccinated guinea pigs, the highest specificity was 84% for FAT followed by ELISA 50%. The lowest specificity estimates was 10% for RBT.

The data suggest that both the FAT and the C- ELISA could distinguished fewer Guinea pigs 4 months post vaccination with B. abortus strain 19 (false positive). These results are similar to the study *(14)* which showed that FAT and C-ELISA could distinguish some elk 4 months post vaccination with B. abortus strain 19. Also, clearance of brucella from vaccinated guinea pigs were examined. Both groups of guinea pigs, the spleens were examined for the presence of brucella organisms 4 months post vaccination. The cultivation on TSA revealed the presence of Brucella organisms in both groups (120 CFU and 80 CFU in Guinea pigs vaccinated with high and reduced doses respectively).

This results are in agreement with that which showed that *(15)* vaccinated bison with brucella abortus strain 19 vaccine, brucella organisms still present in lymph nodes at 16 weeks.

In conclusion, the FAT is the diagnostic test of choice as it shows high sensitivity and specificity in comparison with C-ELISA. It has the capability to distinguish vaccinal antibodies as well as antibodies resulting from exposure to cross reacting organisms from brucella species. Also, it proved that it is easily applicable, adaptable in field and relatively less expensive.

Table 2. A comparison for relative sensitivity and specificity for detection of Brucella species in various herpovarous sera

Animal Species	Brucella isolation		RBT				I-ELISA				C-ELISA				FAT			
	+ve	-ve	+ve	-ve	Sn* %	Sp* %	+ve	-ve	Sn* %	Sp* %	+ve	-ve	Sn* %	Sp* %	+ve	-ve	Sn* %	Sp* %
Cattle	9	7	7	9	81.1	77.7	12	4	75	70	8	8	90	87	9	7	100	100
Sheep	30	24	40	14	75	70	35	29	85.7	82.7	29	29	85.7	82.7	29	25	96.7	96
Goat	17	13	10	20	70.8	65	20	10	85	81.2	8	8	77.2	72.2	16	14	94.4	92.8

Sn* Sensitivity = $\frac{\text{True positive}}{\text{True positive + False negative}}$

Sp* Specificity = $\frac{\text{True negative}}{\text{True Negative + False Positive}}$

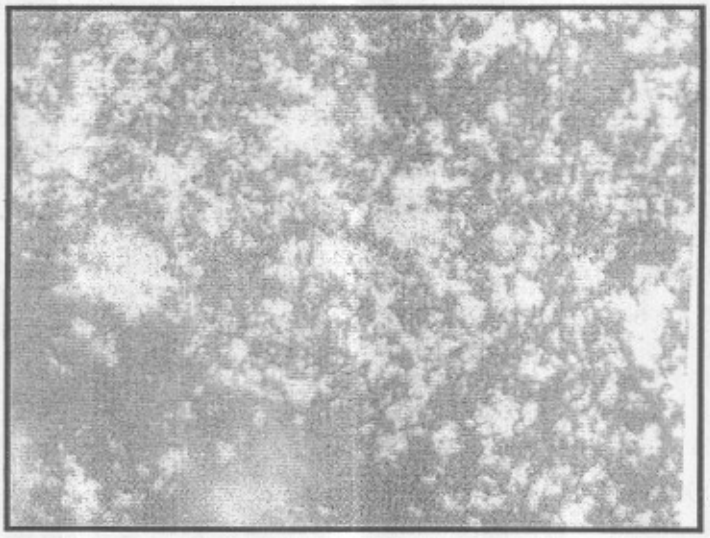

Photo 1. Detection of antibodies against brucella in cattle serum using FAT

REFERENCES

1. ***Adone, R.; Ciuchini, F.; Marianelli, C.; Torantiro, U.; Pistonia, C.; Marcon, G.; Petucci, P.; Francia, M.; Riccordi, G. And Pasquali, P. (2005):*** Rough mutants of Brucella melitensis. Infect. Immun., 73 (7): 4196-4204.

2. ***Nielsen, K.; Gall, D.; Kelly, W.; Vigliocco, A.; Henning, D. and Garcia, M. (1996a):*** Immunoassay development: Application to enzyme immunoassay for diagnosis of brucellosis. Monograph, Agriculture and Agri-Food Canada, Animal Diseases Research Institute, Nepean, Ontario, Canada, 110 pp.

3. ***Kahl, MoMo; McDonagh and Ficht, A. T. (2006):*** Evaluation of protection afforded by B. abortus and B. melitensis unmarked detection mutants exhibiting different rats of clearance in BALB/C mice. Infection and Immunity, July 2996, p. 4048-4057.

4. ***Corner, L.A.; Alton, G.G. and Lyer (1985):*** An evaluation of a biphasic medium for the isolation Brucella abortus from bovine tissue. Aust. Vet. J., 62: 187-189.

5. ***Alton, G.G.; Jones, L.M.; Anhus, R.D. and Verger, J.M. (1988):*** Techniques for Brucella Laboratory Inst. Nat. Rech. Argon, Paris.

6. ***Nielsen, K.; Jolly, Mo; Leishman, Go; Balsevicius, S.; Smith, P.; 'Nicoletti, Po and Thomas, F. (1996b):*** A homogenous fluorescence polarization assay for the detection of Brucella abortus: Journal of Immunological Methods, 195: 161-168.

7. ***EI-Kabbany, M.M. and Zaki, F.F. (2004):*** Trials for preparation and evaluation of peroxidase and fluorescent isothiocyanate conjugated antisera against Equine Influenza virus. Vet Med. J., Giza, 52 (3): 335-346.

8. ***Office International des Epizooties (1996):*** Manual for Standards for Diagnostic Tests and Vaccines. 3rd Edition. Office International des Epizooties, Paris, France, 723 pp.

9. ***Nielsen, K.; Kelly, L.; Gall, D.; Smith, P.; Bosse, J.; Nicolette, P. and Kelly, W. (1994):*** The use of divalent cation chelating agents (EDTA/EGTA) to reduce non-

specific protein interaction in enzyme immunoassay. Vet. Res. Commun., 18: 433-437.

10.Rorald, D. Smith (1991): Veterinary Clinical Epidemiology. Butter-Heinemeinn, Boston London, Oxford Singapore Sydney Toronto,Wellington.

11.Rogers, R.J.; Cook, D.R.; Ketterer, P.J.; Baldock, F.C.; Blackall, P.J. and Stewart, R. W. (1989): An evaluation of three serological tests for antibody to Brucella suis in pigs. Australian Vet. J., 66: 77-80.

12.Angus, R.D. and Barton, C.E. (1984): The production and evaluation of a buffered plate antigen for use in a presumptive test for brucellosis. Developments for Biological Standardization, 56: 549-556.

13.Nielsen, K. (1990): The serological response of cattle immunized with Yersinia enterocollitica O:9 or O: 10 to Yersinia and Brucella abortus antigens in enzyme immunoassays. Vet. J. Immunology and Immunopathology, 24: 373-382.

14.Gall, D.; Nielsen, K.; Forbes, L.; Cook, W.; Leclair, D.; Balsevicius, S.; Kelly, L.; Smith, P. and Mallory, Mo (2001): Journal of Wildlife diseases, 37 (1),2001, pp. 110-118. Wildlife Disease Association.

15.Olsen, S.C.; Cheville, N.E.; Kankle, R.A.; Palmer, M. V. and Jensen, A.E. (1997): Bacterial survival lymph node pathology and serological responses of bison vaccinated with Brucella abortus strain RB51 or strain 19. J. Wild. Dis., 33 (1): 146-151.

الملخص العربي

إقتران الليبوبولي سكاريد أنتيجين بالفلورسين المشع لاستخدامه في تشخيص مرض البروسيلا

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حيث إن مرض البروسيلا من أخطر الأمراض التي تصيب الإنسان و الحيوان فقد أجريت تلك الدراسة لمقارنة مدي حساسية و خصوصية الاختيار الفلورسنتي مع الاختبارات السيرولوجية الأخرى (اختبار الروزبنجال و اختباري الاليزا التنافسي و المثبط) و ذلك في كل من الأرانب الهندي و بعض حيوانات المزرعة (الماشية و الخراف و الماعز). و قد وجد أن الاختبار الفلورسنتي المحضر أعطي نسبة حساسية و خصوصية أعلي في كل من الأرانب الهندي و حيوانات المزرعة مقارنة بالاختبارات السيرولوجية الأخرى. ففي الأرانب الهندي كانت نسبة الخصوصية (100% ،96%، 92.8%) في الأبقار و الخراف و الماعز علي التوالي. أما نسبة الحساسية فكانت 100% ، 96.7 %، 94.4% في الأبقار والخراف و الماعز علي التوالي.

و عليه نستخلص من تلك النتائج أن الاختبار الفلروسنتي هو الأمثل لتشخيص مرض البروسيلا مقارنة باختبارات الاليزا و الروزبنجال و يتطلب ذلك التوصية بإنتاج هذا المستحضر للتشخيص محلياً.