Investigation of antibodies of *Brucella melitensis* in sheep

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Summary

The study was conducted to estimated 192 sheep serum samples for present of antibodies of *Brucella melitensis*. The result was 98 (54.4%) of serum positive when use Rose Bengal test (RBT) while 124 (68.8%) positive when enzyme linked immunesorbant assay (ELISA) test was used.

Also the study showed that 74 (75.5%) and 24 (24.4%) in female and males positive in RBT respectively. While 96 (77.4%) and 28 (22.5%) in females and males positive in ELISA respectively.

Introduction

Brucellosis caused by infection with *Brucella melitensis* is an important zoonosis [1]. Sheep and goat brucellosis (excluding infection which is not pathogenic for human) is a zoonotic infection with important effects on both public health, animal health and production and is wide spread in many areas of the world, particularly in some Mediterranean and middle eastern countries [2] .

In Iraq brucellosis, is still an endemic serious disease among domestic animals and human in spite of the attempt that were implemented in the country to control the disease [3] . In sheep and goat ,the major clinical sign is abortion but other signs may be observed , such as orchitis, epidymitis, hygroma,

arthritis, metritis, and subclinical mastitis . animals may develop self-limiting infections or become latent carrier with potential excretion of the bacteria. This stage is frequently associated with a persistent infection of the mammary gland, supramammary and genital lymph nodes with shedding of the organism in milk and genital secretions .Abortion usually occur late in gestation in goats [4].

Various serological tests have been used for the diagnosis of brucellosis, the most common tests used are serum agglutination test ,coombs anti-brucella test , rose Bengal test and complement fixation test [5].Unequivocal diagnosis can be made only by the isolation and identification of Brucella organism from abortion materials (foetal stomach content and cotyledons),milk and vaginal discharge[6,7],but it is not always practical and possible ,and bacterial culture results are often negative for infected animals [7,8,9].Therefore ; it often necessary to resort to serological tests to identify the specific antibodies in the presence of Brucella antigen [7,8].serological tests can be divided broadly into two groups :screening tests such as RBT ,which is a spot agglutination technique, because it does not need special laboratory facilities and it is simple and easy to perform .It is used to screen sera for Brucella antibodies [10].And confirmatory tests such as ELISA. The present study conducted for detection of Brucella antibodies in sheep sera in Basra province by using RBT and ELISA tests and for comparison between them .

Materials and methods

Sera samples from 180 sheep (54 males and 126 females) slaughtered in the Basrah slaughter house was tested from January 2008 to May 2008 using commercial kit for Rose Bengal Test (Biomerieux, France)and ELISA test

according to (Bahr,et al) [11]. The statistical analysis were made using the qi-square (X^2)test.

Results

Table (1) showed that 98 sera (54.4%) were positive in RBT and 124 sera (68.8%) were positive in the ELISA test .On other hand ,82 sera (45.5%) were negative in the RBT but 56 sera (3.11%) were negative in the ELISA .

The highest percentage of positive results were detected by ELISA (68.8%) in compared to RBT (54.4%).

Table (2) show that the highest rate of positive results in both RBT and ELISA test was in female's sera (75.5% , 77.4% respectively) in compared to male's sera (24.4% ,22.5%) .

Table (1) comparison of RBT and ELISA results in 180 serum sample

Test	No. of positive case	percentage	No. of negative case	percentage
RBT	98	54.4	82	45.5
ELISA	124	68.8	56	3.11

Table (2) The result of RBT and ELISA test in male and female

Test	Female		Male		Total
	No. of positive case	Percentage %	No. of positive case	Percentage %	
RBT	74	75.5	24	24.4	98
ELISA	96	77.4	28	22.5	124

Discussion

RBT is considered as a survey test to investigate the infection occurrence for its high sensitivity ,it can detect the infection even with the lowest antibodies titers and detect the infection in it's early stage , this is due to that the IgM antibodies are the only predominant antibodies during the acute phase of the disease and more active than IgG.[12,13].

According to present results , there is significant difference ($P < 0.05$) between females and males in the percentage of positive results of RBT (75.5% ,24.4% respectively) and ELISA (77.4% ,22.5% respectively) .The explanation of this discrepancy is based on the resistance to infection depends on age , sex ,stage of pregnancy and natural resistance to Brucella which may influence the progressive of infection .Pregnant females are more likely to become infected than non-pregnant females or males .This is because a gravid uterus sustains growth of the organism .On other hand in the male ,localization of the organism in the reproductive organs generally results in the shedding of Brucella in the semen .However ,when used for natural mating ,the risk seems low that infected males transmit the disease to susceptible females .Furthermore ,the course and incidence of the disease is also influenced by natural resistance to infection with' Brucella [14,15].

The standard RBT and complement fixation tests(CF) are the main serological tests used to detect antibodies against *B. abortus* and *B.melitensis* infection [15].In case of making a comparison between results of both serological tests ,our result was in agreement with [16] who found a positive result in each RBT, bacterial isolation and ELISA test 69% ,5% and 100% respectively .Also the results agreement with line of [17], they reported that when testing the sera

from 219 *B.melitensis* culture positive sheep ,both RBT and ELISA tests were more sensitive 98.6% and 96.8% respectively than RBT and CF test 95.0% ,92.7% respectively .

References

1. Thrusfield, M., (1995). Veterinary Epidemiology. Blackwell Scientific. United Kingdom.
2. Alton, G.G. ; Nielsen, K. and Buncan, J.R. (1990). *Brucella melitensis* . In "Animal brucellosis". CRC press. Boston. 383-409.
3. Abed-Mohammed, K.I. (1998). Immunological, Biochemical and Bacteriological study of *Brucella* disease in human . PhD. Thesis, collge science , university of Al-Mustansiriya.
4. SANCO. C. (2001). Brucellosis in sheep and goats (*Brucella melitensis*). European Commission. Scientific committee on animal health and animal welfare. P: 68.
5. Orduña, A., Almaraz, A., Prado, A., Gutiérrez, M.P., García-Pascual, A., Dueñas, A., Cuervo, M., Abad, R., Hernández, B., Lorenzo, B., Bratos, M.A., Rodríguez-Torres, A. (2000). Evaluation of an immunocapture-agglutination test (Brucellacapt) for the serodiagnosis of human brucellosis. J. Clin. Microbiol., 38, 4000-4005.
6. Robinson, A. Guideline for coordinated human and animal brucellosis surveillance. FAO, animal production and health division. 2003.
7. OIE. Manual of Diagnostic tests and vaccines for terrestrial animal : chapter 2.3.1: Bovine brucellosis. 2004. http:/www.oie.int.
8. Clavijo, E. ; Diaz, R. ; Anguita , A. ; Garcia , A.P. and Smits H.L.(2004) Comparison of a dipstick assay for detection of Brucella-specific immunoglobulin M antibodies with other tests for serodiagnosis of human brucellosis. Clin. Diag. Lab.Immunol. 10(4) :612-615.
9. Fostage , G.T., Adesiyun , A.A. ; Hird , D.W. and Hietala, S.K.(2006). Likelihood ratio estimation without a gold standard : A case study evaluating a brucellosis c-ELISA in cattle and water buffalo of Trinidad. Preventive veterinary medicine. 75: 189-205.

10. Alton , G.G. ; Jones, L.N. ; Angus , R.D. and Vergen , J.M. (1988). Techniques for the brucellosis laboratory . In stitute De La recherché agronomique 147 , rue de I' university. 75007 Paris.

11. Bahr,G.M.;RooK,W.A.; Moreno,E. and Lydyard ,P.Z.(1980).Use of the ELISA to screening for micro globulin antibodies .Immunol.41:865-873.

12. Althawani , A.; Al-Bayati , S.; Abass , A. and Abdul-Hussin , T. (2000). A study in the epidemiology of Brucellosis in some production animal in the province of Baghdad. Vet. J. 10(1) : 168-174.

13. Hellman , E. ; Stank , C. and Bauman , M. (1984). Bovine brucellosis among two different cattle populations in Bahred Ghazel province of southern Sudan. Tropen medizien and parasitologic 35: 123-129.

14. Crow ford ,R. P. ; Huber , J.D. and Adams ,B.B. (1990). Epidemiology and surveillance Animal Brucellosis. 131-151 ; 91 ref.

15. Carin-bustuji , B. , Blasco , J.M. ; Gryon , M. and Verger , J.M. (1998). Brucella melitensis infection in sheep : present and future. Vet. Res. 29 :255-274.

16. Araj , G.F. (1986). Evolution of ELISA in the diagnosis of acute and chronic brucellosis in human beings. J. Hyg. Comb. , 97 : 45-69.

17. Morgan.W.J.(1967).The serological diagnosis of bovine brucellosis .Vet .Rec. ,80 :612-620.

الكشف عن الأجسام المضادة لجرثومة *Brucella melitensis* في الأغنام

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الخلاصة

تضمنت الدراسة تقدير وجود الأجسام المضادة لجرثومة *Brucella melitensis* في 192 عينة مصل من الأغنام المأخوذة من مجزرة البصرة ، وكانت النتائج كالآتي ، 98 (54,4%) عينة موجبة عند استخدام فحص البنكال الوردي بينما كانت 124 (68,8%) عينة موجبة عند استخدام فحص الاليزا.

كما وجدت الدراسة أن 74 (75,5%) عينة موجبة في الإناث و 24 (24,4%) عينة موجبة في الذكور عند استخدام فحص البنكال الوردي بينما عند استخدام فحص الاليزا كانت النتائج 96 (77,4%) و 28 (22,5%) عينة موجبة في الإناث والذكور على التوالي.