



The prevalence of brucellosis of farm animals using serum- and milk- ELISA test in Al- Najaf province

Abdulameer A.Hatem

Faculty of Veterinary Medicine/University of Kufa Abdula.hatem@uokufa.edu.iq

07702516689

Abstract

A total of 412 milk and sera samples were collected from the same animals of 88 buffaloes, 212 cattle, 84 sheep and 28 goat were screened by indirect ELISA (i ELISA) milk confirmed with serum i- ELISA. The results of seropositive i-ELISA were 121(29.36%) from all farm animals and distributed to 20 (22.27%) buffaloes, 36 (16.98%) cattle , 56(66.6%) sheep and 9 (32.14%) goat . The positive results of milk i ELISA were 89 (21.6%) from all farm animals and distributed to 8 (9.09%) , 32 (15.09%) , 44(52.83%) and 5(17.85%) represent buffaloes , cattle , sheep and goat respectively . epidemiological informations of animals collected by questionnaire about (abortion , stillbirth and previous history vaccination against brucellosis) . The prevalence was relatively higher in sheep and goat followed by buffaloes and cattle . The sera and milk i ELISA test is only the best serological tests and has a high efficiency and accuracy in the diagnosis and inexpensive and can be used to control of the disease.

Introduction

Brucellosis is a zoonotic disease which is widely spread in the world. It's importance come from its major impact on both public health and farming economy . *Brucella abortus* (bovines) or *Brucella melitensis* (small ruminants) is the most common cause of the disease which can cause abortions as well as it can be excreted by milk (1,2).

Laboratory tests used in diagnosis of brucellosis are culture and detection. Culture is the gold standard diagnostic test, this method is reliable and definitive, but it is unfavorable due to the long time required for isolation and the zoonotic nature of the organism which is a potential hazard for laboratory personnel, while detection of brucella DNA is more simple and sensitive than culture method (3). Other tests are serological tests for presence of antibodies in blood, milk, whey, vaginal mucus and seminal plasma.

The spread of this disease led the health ruling authorities to imply certain screening programs to detect and eradicate suspected cases . screening is usually done by certain serological tests (Rose Bengal Test, Wright's Serum Agglutination Test, Complement Fixation Test, Ring-Test and ELISA) , which are the only used to mass screening systems (3).

The diagnosis of brucellosis is usually done by using indirect ELISAs (iELISA), such method involve immobilization of one of the active components on a solid phase, and iELISAs the antigen bound to a solid phase is usually a polystyrene microtitre plate so that antibody.

Because of the good correlation between isolation of the causative microorganism (brucella) and positive tests obtained from sera and milk samples, Serological test is considered as the more reliable and economical method of diagnosis. When *Brucella* antibodies detection in both milk and serum is considered, the main methods for detecting infected herds are serological tests (4). The Rose Bengal plate test (RBPT) is the most famous and widely used screening test for detection of infection in humans and animals because it's easy to do and simple to read ; however, personal experience can affect the interpretations of results (5). Because of the false positive and false negative results of the RBPT and MRT, recently, several researchers reported the usage of Enzyme-linked Immuno Sorbent Assay (ELISA) for *Brucella* antibody in ruminant serum or milk (6,7,8,9). So the indirect ELISA is



the more specific and sensitive methods that can be applied to process a lot of samples in a short time.

The Aim of the study

- (a) a comparison between prevalences of the disease in different livestock species in Al-Najaf province.
- (b) provide a baseline data for further study .
- (c) identification of anti-Brucella antibodies in buffalos , cattle , sheep and goat serum and milk samples.
- (d) to find a starting point to control spread of the disease.

Materials and Methods

The study accomplished through the period between May to August on five different regions representing all over Governorate of Al-najaf . A total of 412 sera and milk samples were collected from farm animals of different species included 88 buffaloes , 212 cattle ,84 sheep and 28 goat were selected for this study. The individual animals were sampled at the same time for both serum / milk (from each one). The positive serum/milk samples were obtained from permanently infected flocks, where clinical symptoms (abortions) occurred .Samples of serum and milk were taken from buffaloes and cattle which aren't vaccinated against *Brucella* ,while sheep and goats with previous history of vaccination.

Collection of blood sample :

By Jugular venipuncture, samples of 10 ml blood were taken from a healthy looking animals by using needles, needle holder and plain vacuotainer tubes . To separation of serum ,samples were kept at 4°C overnight then centrifuged at 3000 rpm for 10 minutes. The collected sera coded and then kept at -20°C to be ready to use at the time of test.

Collection of milk samples

The collection of milk samples was done under aseptic technique. the teat cleaned well with a cotton soaked in 70% ethyl alcohol. After discard of the first three streams of milk , 10 ml of milk was taken in a sterile glass bottle then Samples were kept in a box filled with ice while transported immediatly to Al-najaf veterinary hospital's laboratory to be analysed directly.

Indirect ELISA test for milk samples

the test procedure and kits were applied according to ID screen brucellosis milk indirect elisa kit – ID vet – grabeis- France (BRUMILK ver 0512GB) .

ELISA test for serum

The test kits and procedure were supplied by serelisa brucella kit-synbiotic-Europe Code: ASBRU3OCB.

Results and discussion

An indirect enzyme-linked immunosorbent assay (i-ELISA) was validated to detect *Brucella* antibodies in sera & milk of buffaloes , cattle , sheep and goat. It is preferable to use LPS-based ELISA. The latter is more accurate & less cross-reactions in determining *Brucella* antibodies (10).



A total of 412 serum and milk samples were analyzed from five regions in Alnajaf province . Among 412 farm animals (88 were buffalos , 212 were cattle , 84 were sheep and 28 were goat) , 121 (29.36%) were found reactive positive serum ELISA Table (1) and 89 (21.6%) were reactive positive milk ELISA table (2) . Among the tested animals the serum prevalence of brucellosis in farm animals as follow : 22.7% buffaloes , 16.98% cattle , 66.6% sheep and 32.14% goat showed reactivity for brucellosis (table 1).

Table (1): Number and percentage of positive samples of sera i- ELISA test in different farm animals

Species	of samples	of Positive serum	Percentage
Buffalo	88	20	22.72
Cattle	212	36	16.98
Sheep	84	56	66.6
Goat	28	9	32.14
Total	412	121	29.36

The highest percentage of positive results were detected by serum ELISA (29.36%) in compared to milk ELISA (21.6%).

Table (2). Number and percentage of positive samples of milk i- ELISA test in different farm animals

Species	No. of samples	No. of Positive	percentage
Buffalo	88	8	9.09
Cattle	212	32	15.09
Sheep	84	44	52.83
Goat	28	5	17.85
Total	412	89	21.6

The sensitivity of sera and milk ELISA test were 88.8% , 78.57 % and 40% of cattle , sheep and buffalo respectively table (1) , whereas specificity 100% in all farm animals .

The prevalence of was much higher in buffaloes than cattle of serum i-ELISA test 22.7% and 16.98% respectively , while contrary in milk i-ELISA test 9.09% ,15.09% in buffaloes and cattle respectively , the interpretation of these results are using artificial insemination in cattle ,in opposite buffaloes using natural mating, the risk is somewhat low that infected males transport the infection to vulnerable females . Also fatty milk of buffaloes make it less sensitive than serum (sensitivity 40%). In cattle colonization of the udder is frequent and excretion of brucella in the milk may be prolonged for months or years, this lead to increase production of antibrucella antibodies (sensitivity 88.8%). Also, the contact between different herds/flocks can increase the spread of disease to susceptible animals accordance with (11). The percentage of positive serum i-ELISA test of cattle in blood samples subjected to was 16.98% , less than (12) 20.45 % in Iraq.

The sensitivity and specificity of i-ELISA were 88.8 % and 100% respectively . Although the positive percentages of *Brucella* antibodies in milk samples tested in this study was found 15.09 % lower than (13, 14)and its higher than (15) . Its considered as high for Alnajaf state, where no previous history of vaccination against brucellosis is applied in cattle.

The reason for the differences in infection rates between this study and others is that samples may be taken randomly from the area that disease is endemic and other free of the disease , or the density of livestock , moreover cross reaction of lipopolysuccaride of other gram negative bacteria (16).



In sheep the result of indirect ELISA test (i-ELISA) total seroprevalence was (66.6%) , in line with (17) 23.6% -100% was recorded in Mosul and (18) in Basrha (68.8%), but higher than (19) (29.34 %) , the result of milk i-ELISA test in sheep (same flock) were 52.83% , in reverse with (20) in Iraq. This variation in results may be due to differences in geographical region , age of animals and previous history of vaccination against *Brucella* in sheep and goats , subsequently cause increase titration of antibodies in serum , serological tests, can distinguish between antibodies that arise from infection and those from vaccination, respectively, have not been developed accordance with (21). if the lactating udder is infected, less serological response will occur, and localization is confined to a few number of lymph nodes may fail to produce immunological response at all species .

The prevalence of caprine seroprevalence brucellosis was 32.14% , less than presented by (22 , 23) and 17.85% of milk I-ELISA test , supported by (3,24) in Iraq , the level of antibodies against brucella decline in milk after parturition , while remain high level in blood .

Although followed planned program of vaccination against brucellosis in small ruminant in Iraq , the disease still endemic , because of movement of animals through Alnajaf deserts . In sheep, goats and cattle, The Seroprevalence of disease was widely & irregularly distributed among flocks and provinces ,which refer by (25,26).

The positive reactor of serum and milk i-ELISA test was 29.36% and 21.6% respectively in all farm animals ,the reason may be due to that test is accuracy and efficiency in detecting all immunoglobulins in serum accordance to (27).

This assay does not distinguish between infected & vaccinated animals, however, it can be used for free monitoring in vaccinated areas and for diagnosis in affected areas where animals were vaccinated more than one year before.

The I-ELISA kit evaluated in our laboratory seemed to be rapid, simple, sensitive, and specific for detecting antibodies to *Brucella* . The I-ELISA should also be evaluated as a diagnostic tool in control programs in Iraq.

Conclusion and recommendations :

Conclusions :

1. The Bruc ELISA test is a sensitive, specific, and inexpensive method for screening large numbers of individual or bulk milk samples for antibody to *B. abortus*.

Recommendations :

1. Test and reduction of reservoir of infection and removes infected animals from the herd and reduces exposure to causative agents and transmission within the herd.
2. Use of effective vaccination program to reduce of economic losses .



References

1. Al-Khalaf, S.A., Mohamad, B.T., Nicoletti, P., 1992. Control Of Brucellosis In Kuwait By Vaccination Of Cattle, Sheep And Goats With *Brucella abortus* Strain 19 or *Brucella melitensis* strain Rev.1.Trop.Animal health Prod. 24, 45-49.
2. Alton, G.G. ; Nielsen, K. and Buncan, J.R. 1990. *Brucella melitensis* . In"Animal brucellosis". CRC press. Boston. 383-409.
3. Nielsen K. Diagnosis of brucellosis by serology. Vet Microbiol 2002; 90:447--459.
4. Blasco, J.M., Marín, C.M., Jiménez De Bagüés, M.P., Barberán, M., Hernández, A., Molina, L., Velasco, J., Díaz, R., Moriyón, I., 1994. Evaluation Of Allergic And Serological Tests For Diagnosis Of *Brucella melitensis* in sheep and goats. Vet Rec ; 134: 415-420.
5. Noriello S 2004. Laboratory-acquired brucellosis- Detection of *Brucella abortus* in Bovine Milk by Polymerase Chain Reaction. Emerg. Infect. Dis., 10: 1848-1850.
6. Cho D, Nam H, Kim J, Heo E, Cho Y, Hwang I, Kim J, Kim J, Jung S 2010. Quantative Rose Bengal Test for diagnosis of bovine brucellosis. J. Immunoassay Immunochem., 31 (2): 120-30
7. Hunter D, Allen J 1972. An evaluation of milk and blood tests used to diagnose brucellosis. Vet. Rec., (13):310-312
8. McGiven, Tucker J, Perrett L, Stack BS, MacMillan A 2003. Validation of FPA and cELISA for the detection of antibodies to *Brucella abortus* in cattle sera and comparison to SAT, CFT and iELISA. J. immunol. 278: 171-17.
9. OIE, Office International des Epizooties (World organization for Animal Health) (2004). Bovine Brucellosis . Section 2.3 in OIE manual of Standards for diagnostic tests and vaccines. 5th ed. OIE Paris.
10. Patel T (2007). Serological, cultural and molecular detection of *Brucella* infection in bovine including quantification in milk by real time PCR. Masters thesis, College of Veterinary Science and Animal Husbandry, Aand Agricultural University, Anand.
11. Neilsen K, Smith P, Gall D, Perez B, Cosma C, Muller P, Trottier J, Cote G, Boag L, Bosse J1996: Development and validation of an indirect enzyme immunoassay for detection of antibody to *Brucella abortus* in milk. Vet Microbiol, 52, 165-173,.
12. Crespo, F. 1994 Brucellosis ovinary caprina.OIE, Paris, France.
13. Idrees B. A. Al-abdaly 2012.A comparative study of Rose Bengal and indirect ELISA tests for detection of the brucella antibodies in sera of cows in some areas of Salah El-Din province, Journal of Tikrit Agriculture science 12 .No.4.
14. Göknur Terzi , Özlem BÜYÜKTANIR , Oktay GENÇ , Ali GÜCÜKOĞLU , Nevzat YURDUSEV. 2009 Detection of Brucella Antibody and DNA in Cow Milk by ELISA and PCR Methods. Kafkas Univ Vet Fak Derg (Suppl-A): S47-S52, DOI:10.9775/kvfd..1284.



15. Ahmed, T.M., Bassiony.M.M . and Ibrahim , I.G.A. 2002: Field evaluation of complement fixation test and enzyme immunosorbent assay for detection of brucellosis in Bull.J.Egypt.Vet.Med.Assoc, 62(1) :119-125.
16. Amin KMR, Rahman MB, Rahman MS, Han JC, Park JH, Chae JS. 2005 Prevalence of Brucella antibodies in sera of cows in Bangladesh. J Vet Sci , 6, 223-226.
17. Fosgate, 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. Preven. Vet. Med. , 75: 189-205.
18. Arslan S. H., Hussain Kh. J., Asmaeel S. A. and Hassan S. D.. 2009 Comparison between Rose Bengal and indirect ELISA tests for detection of the antiBrucella antibodies in serum of sheep in Mosul city Iraqi Journal of Veterinary Sciences (21), 4, 108-113.
19. Abeer L. Mohammad; Esraa Ab.W. Mohammad Ali; Wesam M. Salih 2009. Investigation of antibodies of Brucella melitensis in sheep in Basra Vet.J. 11, 46-48 .
20. Esmaeel S. A., Mohmmmed B. A., Hassan S. D. and Hassain Kh. J. 2010. Detection of anti brucella antibodies in lambs and goat kids using rose bengal test and indirect ELISA in Gugjeli - Ninavah province, Iraq. Iraqi Journal of Veterinary Sciences, Vol. 24, No. 2, (23-26).
21. Al-Obaidi Q. T., Hassan S. D., Mohammad B. A. and Arslan S. H. 2009. Prevalence of brucellosis using indirect ELISA test in raw milk in individual cases of ewes and does in Mosul city Iraqi Journal of Veterinary Sciences, Vol. 23, No. 2, (111-114).
22. Abalos P., Ibarra, L., Pinochet, L., NAVIA, F., Boisier, X., (1996) : Residual antiBrucella abortus strain 19 antibodies detected in adult cattle by two indirect-ELISA tests, Veterinary Record 138, 140.
23. Hussein AAA, Sayed ASM, El Feki MA. 2005 Seroepidemiological study on human brucellosis in Assiut Governorate. Egypt J Immunol;12:49-56.
24. Gulaand ST, Khan A. Epidemiology and Epizootology of brucellosis 2007: A review. Pakistan Vet J.;27(3):145-151.
24. H.A. Kaoud¹, Manal.M. Zaki¹, A.R. El-Dahshan¹ , Shima¹ .A. Nasr¹ 2010. Epidemiology of Brucellosis Among Farm Animals natural and science.;8(5)
25. Al-Ani FK, El-Qaderi S, Hailat NQ, Razziq R, Al- Darraji AM. 2004 Human and animal brucellosis in Jordan between 1996 and 1998: a study.Rev.Sci.Tech.23(3):831-40.
26. Muma JB, Samui KL, Siamudaala VM, Oloya J, Matop G, Omer MK, Munyeme M, Mubita C, Skjerve E. 2006. Prevalence of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia. Trop Anim Health Prod . 2006 Apr; 38(3):195-206.
27. Gall, D. and Nielsen , K. 2004 . Serological diagnosis of bovine brucellosis : A review of test performance and cost comparison. Rev. Sci. tech. Int Epiz. , 23 : 989-1002.