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Evaluation of efficacy of some serological tests used for diagnosis of brucellosis in cattle in Egypt using latent class analysis

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Abstract

In this study serum samples were collected from 4 different groups of cattle, Group I (non-vaccinated *Brucella* infected group), Group II (Vaccinated *Brucella* infected group), Group III (Non-vaccinated *Brucella* free group) and Group IV (vaccinated *Brucella* free group). These samples were subjected to the different serological tests including Rose Bengal plate antigen test, Tube Agglutination test, Rivanol test, Indirect Enzyme Linked Immunosorbent Assay and Competitive Enzyme Linked Immunosorbent Assay. Statistical analysis of the obtained results in different cattle groups was carried out using Latent Class Analysis (Lem model). The prevalence of brucellosis was 6.4%, the sensitivity of RBPT was 96.1% while its specificity was 99.3%, the sensitivity of Rivanol test was 85% while its specificity was 100%, the sensitivity of Indirect Enzyme Linked Immunosorbent assay was 100% while its specificity was 98.3 % and the sensitivity of Competitive Enzyme Linked Immunosorbent assay was 97.1% while its specificity was 100%. The results proved that, the most sensitive test was Indirect Enzyme Linked Immunosorbent assay while the most specific test was Competitive Enzyme Linked Immunosorbent assay. This study therefore, recommends the use of Indirect Enzyme Linked Immunosorbent assay as a screening test and Competitive Enzyme Linked Immunosorbent assay as a confirmatory test. Bacteriological examination was carried out on supramammary lymph nodes and spleen of some slaughtered seropositive cattle, the rate of isolation was 25% from non-vaccinated infected group and 10% from vaccinated infected group. *Brucella melitensis* biovar3 was recovered only from supramammary lymph nodes.

Keywords: Brucellosis, Cattle, Sensitivity, Serology, Specificity, Latent Class Analysis

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Introduction

Brucellosis is one of the important bacterial diseases affecting cattle, causing severe economic losses in the form of (abortion, loss of milk production and infertility); in addition it has severe zoonotic importance (Nielsen & Duncan, 1990).

In Egypt, there is no clear well-established national policy for the disease control among cattle, this is partly because, most of cattle populations are raised in small numbers (2-3 cattle raised by a farmer) with some exceptions of large numbers of cattle kept in herds in organized farms which do not exceed 10% of cattle population. There is no mandatory vaccination against bovine brucellosis all over the country. Different vaccines as strain 19 and RB51 are used in individual bases in some farms and even both vaccines are used in the same farm.

Brucella melitensis proved to be the endogenous strain in cattle, buffaloes, sheep and goats; this can be attributed to raising sheep and goats with cattle or buffaloes in villages. Most sheep or goat flocks in Egypt are mobile and movement of infected sheep or goats can contaminate pastures and spread brucellosis to other animals. Using of S19 *Brucella abortus* vaccine and occasional use of RB51 (rough

strain of *Brucella abortus* vaccine) is the main cause of domination of *Brucella melitensis* rather than *Brucella abortus* among different animal species in Egypt (Alton *et al.,* 1988; Corbel, 2006).

Diagnosis of brucellosis depends on either direct diagnosis through isolation and identification of the causative microorganisms from infected animals showing abortion, stillbirth and retained placenta or indirect diagnosis through the using of serological tests such as [Rose Bengal (RBPT), Rivanol, Tube agglutination test (TAT), indirect Enzyme linked Immunosorbent Assay (iELISA) and competitive Enzyme linked Immunosorbent Assay (cELISA)]. The problem in diagnosis of brucellosis in Egypt is that, there is no clear data for most vaccinated herds, also the haphazard use of different vaccines in the same farm and overlapping between free and infected areas.

In this study, Latent Class Analysis (LCA) used to evaluate the efficacy of different serological tests in absence of gold standard in different cattle groups in the form of determination of sensitivity and specificity of each serological test and also the prevalence of the disease.

Materials and methods

Four hundred adult dairy cattle age 3-6 years were used in the study. These cattle were classified into 4 groups, Group I (non-vaccinated *Brucella* infected group), Group II (vaccinated *Brucella* infected group), Group III (non-vaccinated *Brucella* free group) and Group IV (vaccinated *Brucella* free group). A total of 400 serum samples (100 samples from each group) were collected according to MacMillan (1990). Samples for bacteriological examination were obtained from spleen and supramammary lymph nodes of 26 slaughtered seropositive cattle. Antigens for Rose Bengal, Tube Agglutination and Rivanol tests were obtained from Veterinary Serum and Research Institute, Abassia, Cairo, Egypt. Reagents used for Indirect Enzyme Linked Immunosorbent Assay and Competitive Enzyme Linked Immunosorbent Assay were kindly obtained from Veterinary Laboratory Agency, New Haw, Addleston, Surrey KT15 3NB United Kingdom.

Clinical examination was carried out to detect clinical findings suggestive for brucellosis in different cattle groups while serological diagnosis was carried out on serum samples using Rose Bengal test, Tube Agglutination Test and Rivanol test according to Alton *et al.* (1988). Indirect Enzyme Linked Immunosorbent Assay and Competitive Enzyme Linked Immunosorbent Assay according to OIE (2008). Bacterial isolation and identification was carried according to Alton *et al.* (1988)

Statistical analysis was carried out using Latent Class Analysis (LCA) to determine the prevalence of the disease, sensitivity and specificity of each test used in the absence of gold standard. This was carried out by Lem Statistical Model according to Vermunt (1997).

Result

The rate of *Brucella* infection was shown in table 1. Group I examined serologically using RBPT as screening test revealed that 17% of the examined cattle were positive and 83% were negative, using TAT for confirmation of the for mentioned seropositive samples 16 animals out of 17 seropositive samples were positive. The serologically positive cattle were subjected to bacteriological examination of samples obtained from spleen and supramamary lymph nodes. *Brucella melitensis* biovar3 was isolated only from supramamary lymph nodes of four cattle. Group II examined serologically using RBPT as screening test revealed that 10% of the examined cattle were positive and 90% were negative, using TAT for confirmation of the formentioned seropositive samples, all seropositive samples with RBPT were also positive with TAT. The serologically positive cattle were subjected to bacteriological examination

Brucella melitensis biovar3 was isolated only from supramammary lymph node of only one cattle. Group III examined serologically using RBPT as screening test. All serum samples examined were negative for RBPT. Group IV examined serologically using RBPT as screening test. All serum samples examined were negative for RBPT.

Table 2 showed that, out of the examined 400 serum samples collected from different cattle herds with or without history of brucellosis and/or not vaccinated, 27 samples were positive with RBPT (6.75%), 22 samples were positive with Rivanol (5.5%), 32 samples were positive with iELISA (8%) and 26 samples were positive with cELISA (6.5%).

Statistical analysis of the obtained results in different cattle herds with or without history of brucellosis and/or not vaccinated using latent class analysis (LEM model) table 3 showed that, the prevalence of the disease was 6.4%, the sensitivity of RBPT was 96.1% while its specificity was 99.3%%, the sensitivity of Rivanol test was 85 % while its specificity was 100 %, the sensitivity of iELISA was 100% while its specificity was 98.3 % and the sensitivity of cELISA was 97.1 % while its specificity was 100 %.

Table 1: Rate of *Brucella* infection in different groups

Clinical picture	Group	Number	Vaccination status	Serological status				Bacteriological culture	
				RBPT		TAT			
				Examined	Positive	Examined	Positive	Examined	Positive
Present	I	100	Non-vaccinated	100	17	17	16	16	4
Present	II	100	Vaccinated	100	10	10	10	10	1
Absent	III	100	Non-vaccinated	100	0	-	-	-	-
Absent	IV	100	Vaccinated	100	0	-	-	-	-

Table 2: Results of different serological tests used for diagnosis of brucellosis in all serum samples examined from different cattle herds

Test	No. of examined cattle	No. of positive	%
RBPT	400	27	6.8%
Rivanol	400	22	5.5%
iELISA	400	32	8.0 %
cELISA	400	26	6.5 %

Table 3: Results of sensitivity, specificity and prevalence of brucellosis using different serological tests in absence of gold standard in the examined herds

Test	Sensitivity	Specificity
RBPT	96.1%	99.3%
Rivanol	85%	100%
iELISA	100%	98.3%
cELISA	97.1%	100%

Prevalence of the disease 6.4%

Discussion

The efficacy of any diagnostic test is determined through estimation of the diagnostic sensitivity and specificity. The status of the disease is determined by the prevalence which means the frequency of the disease in a population (Thrusfield, 2003)

Routine serological tests i.e RBPT as screening test and TAT as confirmatory test were carried out according to Stemshorn *et al.* (1985), MacMillan, (1990), Ibrahim *et al.* (1993) and Corbel (2006). The obtained results were confirmed by bacteriological examinations on supramammary lymph nodes and spleen collected from some slaughtered seropositive cattle.

The rate of infection with brucellosis was higher in infected non-vaccinated group (16%) than that vaccinated infected group which reached 10%. Therefore, the study could conclude that, the reduced percentage of infection with brucellosis may be due to calfhood vaccination with S 19 Brucella abortus *vaccine,* and this agree with the results obtained by Alton *et al.* (1988); Corbel, (2006); Peniche *et al.* (2008). All *Brucella* isolates were recovered from supramammary lymph nodes which indicate that the most important site for isolation of brucellosis in cattle is the supramamary lymph nodes. That agrees with the results obtained by Alton *et al.* (1988), Mayfield *et al.* (1990) and Corbel (2006). Bacteriological examination of the samples obtained from supramammary lymph nodes from group I revealed that out of 16 seropositive cattle only 4 (25%) samples were positive bacteriologically while in vaccinated infected group II out of 10 seropositive cattle only one(10%) cattle was positive bacteriologically. We could conclude that bacteriological isolation of *Brucella* has low sensitivity for detection of infected animals which explained why some of the slaughtered seropositive animals failed to reveal any *Brucella* isolates. But Refai (2002), Samaha *et al.* (2008) and Gwida *et al.* (2010) have reported bacteriological isolation of *Brucella* to be highly specific. Negative bacteriological isolation for brucellosis doesn't mean that the animal is not infected and this may be

attributed to either the absence of the bacterium in microorganism, also the process of isolation is not always practicable especially in living animals and microbiological culture take long duration (days to weeks) to produce a result, making the process of isolation impractical for field testing or testing where livestock health authorities must make immediate decisions, (Alton *et al.*, 1988; Refai, 2002; Samaha *et al.,* 2008). All the isolated strains were *Brucella melitensis* biovar3 which is considered as an active *Brucella* infection in tested cattle. These results revealed that the most common isolated strain from cattle was *Brucella melitensis* biovar3. This finding is consistent with reports of *Brucella melitensis*, particularly biovar3, being the main cause of brucellosis in animals and humans in many countries including Egypt, (Nielsen & Gall, 1998) and Samaha *et al.* (2008) who attributed that increased prevalence of *Brucella melitensis* biovar3 in cattle in Egypt is due to raising sheep and goats with cattle or buffaloes in villages, also most sheep and/or goat flocks in Egypt are mobile. Movement of infected sheep and/or goats can contaminate pastures and spread brucellosis to other animals (e.g., cattle or buffaloes) in other herds or areas. This movement is a major risk factor for failure of brucellosis eradication programs. Elimination or control of infection in sheep and goat flocks can reduce spread of the disease in cattle and buffaloes.

Studying the evaluation of the efficacy of RBPT, Rivanol, iELISA and cELISA tests for diagnosis of brucellosis in different groups. Some animals gave negative results in the conventional screening test (RBPT) while it gave positive results with iELISA and/or cELISA tests. It was assumed to be false positive reactors where in fact it may be brucellosis in early stage of infection detected by ELISAs due to their higher sensitivities these were reported by Nielsen & Gall (1998).

The traditional approaches for evaluation of sensitivity and specificity of diagnostic tests comprise presence of gold standard or the use of a reference test (test represented true disease status). The gold standard in diagnosis of brucellosis is the bacterial isolation which is often unsuccessful. This may be attributed to, the absence of the bacterium in the cultured tissues or insufficient numbers of the microorganism, also the process of isolation is not always practicable especially in living animals and the microbiological culture take long duration (days to weeks) to produce a result, making the process of isolation impractical for field testing, (Alton *et al.* 1988; Refai, 2002; Corbel, 2006; Samaha, *et al.* 2008)**.** Therefore we used Latent Class Analysis (LCA) which is a mathematical technique that uses a statistical model to relate unobserved (latent) conditions to multiple diagnostic test results. LCA models the probability of each combination of test results conditionally on the latent class (infected or non-infected). From these probabilities, the sensitivity and specificity of all tests included in the model can be estimated (Boelaret *et al.,* 1999; Goetghebeur, 2000; Bajani *et al*., 2003; Pepe & Janes*,* 2007; Engel *et al.*, 2008).

Statistical analysis of the obtained results in non-vaccinated cattle with high rate of infection (group I) using LCA (Lem model). The higher sensitivity of iELISA test (100%) may be due to the using of polyclonal anti-bovine IgG (H&L) chain specificity conjugate which causes enhanced detection of both IgM and IgG antibodies as reported by Abalos *et al.* (1997) and Poester *et al.* (1997). The principal requirement of any screening assay is that it must be diagnostically sensitive as possible in order to ensure that all true serological reactors are detected. The obtained results revealed that, iELISA test has higher sensitivity (100%) than the other tests (Rivanol, RBPT and cELISA), therefore we can recommended that iELISA test could be used as screening test for diagnosis of brucellosis in non-vaccinated herd with high rate of infection**.** This agrees with the results obtained by Saravi *et al.* (1995), Marino *et al.* (1997) and Nielsen and Yu (2010). The current British Brucellosis Surveillance Strategy uses iELISA method to screen cattle herds "ELISAs are sensitive, detect all stages of the infection, easy to perform, to automate, produce objective results, rapidly lend themselves to Quality Assurance Programmes, and can cut the cost of staff training due to the universality of the ELISA method for different applications" (McGiven *et al*., 2003). The highest specificity in case of cELISA is due to using of specific monoclonal antibodies as a conjugate which has the ability to compete with other non specific antibodies and attach to certain specific epitopes on sLPS antigen (Philo & Edwards (2002); Godfroid *et al.,* (2010); Nielsen & Yu (2010). The highest specificity in case of Rivanol test is due to precipitation of IgM antibodies using Rivanol solution to exclude the non specific reactors and detect only IgG antibodies (Alton *et al*., 1988). Due to high specificity of cELISA (100%) we could recommended it as confirmatory test on seropositive animals using iELISA as a screening test in order to exclude the non specific reactors and this agree with the results obtained by

Marino *et al.* (1997) and Nielsen and Yu (2010). cELISA has both high diagnostic specificity (100%) and sensitivity (98.8%) but Rivanol has high diagnostic specificity (100%) and low diagnostic sensitivity (86.47%). The confirmatory tests must demonstrate high level of diagnostic specificity and maintain effective diagnostic sensitivity in order to decrease the number of false positive reactors to the minimal levels (Saravi *et al.*, 1995; Poester *et al.*, 1997). Therefore cELISA is more efficient than Rivanol test as a confirmatory test in diagnosis of brucellosis in cattle herds with high prevalence of infection.

Studying the evaluation of the different serological tests used for diagnosis of brucellosis in vaccinated herd with high rate of infection (group II), Some animals gave positive results in the conventional test (RBPT) but were negative to iELISA and/or cELISA. It was assumed to be in fact that ELISA failed to diagnose brucellosis. This reaction could be due to the presence of residual antibodies from the use of *Brucella abortus* S19 vaccine, the same results obtained by Nielsen & Gall (1998).

Statistical analysis of the obtained results in vaccinated cattle with high rate of infection (group II) using LCA (Lem model). ELISA technique is a fast diagnostic method that enables a large number of samples to be tested at a relatively low cost. The higher sensitivity of ELISA tests may be due to the ability of ELISA to detect infected animals in early stages of infection and also the ability to detect lower antibody titers (Abalos *et al.,* 1997; Peraza *et al.*, 1997). cELISA gave negative results on some serum samples collected from vaccinated non infected cattle although these serum samples gave positive results by other serological tests (RBPT and iELISA). The positivity of other serological tests from our point of view can be attributed to the remaining of antibody titer against S19 *Brucella abortus* vaccine this result agree with the result obtained by Nielsen & Gall (1998), while the higher specificity of cELISA as mentioned by Philo & Edwards (2002) is due to cELISA is based on labeled mouse monoclonal antibodies that differentially compete with antibodies against Strain 19 vaccination and field-strain of *B. abortus* for a specific antigenic determinant on the outer cell wall of the bacterium.

Antibodies produced in response to Strain 19 vaccine compete poorly with the monoclonal antibody; while antibodies produced following field strain *B. abortus* infection compete strongly with the monoclonal antibody. In the vaccinated infected group II with high prevalence of infection (9%), iELISA and cELISA showed high sensitivity, while cELISA showed also high specificity. This study therefore, recommended the use of iELISA as screening test and cELISA as confirmatory test, the results agree with the results obtained by Saravi *et al.* (1995) who stated that, screening tests must be highly sensitive in order to reduce the number of false negative animals and confirmatory tests must demonstrate high level of diagnostic specificity and maintain effective diagnostic sensitivity in order to decrease the number of false positive reactors to the minimal levels.

The results showed that, among sera collected from the different cattle herds with or without history of brucellosis and/or not vaccinated represented in the 4 previously mentioned groups, the most sensitive test was the iELISA while the most specific test was cELISA**.**

In conclusion, the study could conclude that, the use of iELISA to detect truly infected animals (sensitivity) and cELISA to exclude truly non infected (specificity) appeared to be of most importance. The absence of clear mandatory vaccination program against brucellosis and the use of different vaccines with different doses necessitates the use of these 2 tests together in Egyptian condition.

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References

Abalos P, Pinochet L, Ibarra L & Navia F (1997). Use of an indirect enzyme linked immunosorbent assay for the diagnosis and epidemiological studies of *Brucella abortus* in Chile. Diagnosis and Epidemiology of Animal Diseases in Latin America, Proceedings of the Final Research Co-ordination Meetings of FAO/IAEA/SIDA, Pp 49-54.

Alton GG, Jones LM, Angus RD & Verger JM (1988). Techniques for the brucellosis laboratory. Paris: Institut National de la Recherche Agronomique 147, rue de l'Universite, 1st edition. 75007 Paris, Pp 1-200.

Bajani MD, Ashford DA, Bragg SL, Woods CW, Aye T, Spiegel RA, Plikaytis BD, Perkins PA, Phelan M, Levett PN & Weyant RS (2003). Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. *Journal of Clinical Microbiology*, **41**(2): 803–809.

Boelaret M, Aoun K, Liinev J, Goetghebeur E & Van Der Stuyft P (1999). The potential of Latent Class Analysis in diagnostic test validation for canine *Leishmania infantum* infection. *Epidemiology and Infection*, **123**(3): 499-506.

Bronsvoort BM, Koterwas B, Land F, Handel IG, Tucker J, Morgan KL, Tanya VN, Abdoel TH & Smits HL (2009). Comparison of a Flow Assay for Brucellosis Antibodies with the Reference cELISA Test in West African Bos indicus. *PLoS ONE*, **4**(4): e5221.

Corbel MJ (2006). Brucellosis in humans and animals. WHO Liberary Cataloguing- in- Publication data, produced by WHO in collaboration with FAO of the United States and World Organization of animal health, Pp 28-31.

Engel B, Buist W, Orsel K, Dekker A, Clercq K, Grazioli S & Roermund H, (2008). A Bayesian evaluation of six diagnostic tests for foot-and-mouth disease for vaccinated and non-vaccinated cattle. *Preventive Veterinary Medicine,* **86**(1-2): 124–138.

Goetghebeur E, Liinev J, Boelaert M & Van der Stuyft P (2000). Diagnostic test analyses in search of their gold standard: latent class analyses with random effects. *Statistical Methods Medical Research,* **9**(3): 231-248.

Godfroid J, Nielsen K & Saegerman C (2010). Diagnosis of Brucellosis in Livestock and Wildlife. *Croatian Medical Journal,* **51**(4): 296-305.

Gwida M, Al Dahouk S, Melzer F, Rosler U, Neubauer H & Tomaso H (2010). Brucellosis – Regionally Emerging Zoonotic Disease?. *Croatian Medical Journal*, **51**(4): 289-95.

Ibrahim IGA, Bassiony MM, Farag YA, Shalaby MNH, Kholeaf ZM & Farid A (1993). Evaluation of brucellosis status in low titred bovine reactors. *In: Proceedings of 5th Annual Congress, Egyptian Society of Animal Reproduction and Fertility,* Cairo-Egypt, Pp 218-223.

MacMillan A (1990). *Conventional serological test:* In *Animal Brucellosis (Nielsen K & Duncan JR, editors)*. CRC Press, Boca Raton, Pp 153-197.

Marino O, Rueda E, Sedano L,Calderon C, Ortega A, Puentes A & Zuniga L (1997). Comparative evaluation of competitive ELISA in Colombian cattle. Diagnosis and Epidemiology of Animal Diseases in Latin America, Proceedings of the Final Research Co-ordination Meetings of FAO/IAEA/SIDA, Pp 131-140.

Mayfield JE, Bantle JA, Ewalt DR, Meador VP & Tabatabai LB (1990). Detection of brucella cells and cell components In*: Animal Brucellosis*. *Nielsen K & Duncan JR, editors)* CRC Press, Boca Raton, Pp 97 - 120.

McGiven JA, Tucker JD, Perrett LL, Stack JA, Brew SD & MacMillan AP (2003). Validation of FPA and cELISA for the detection of antibodies to *Brucella abortus* in cattle sera and comparison to SAT, CFT and iELISA. *Journal of Immunological Methods*, **278**(1-2): 171-178.

Muma JB, Toft N, Oloya J**,** Lund A, Nielsen K, Samui K & Skjerve E (2007). Evaluation of three serological tests for brucellosis in naturally infected cattle using latent class analysis. *Veterinary Microbiology,* **125**(1-2): 187–192.

Munoz PM, Marın CM, Monreal D, Gonzalez D, Garin-Bastuji B & Mainar-Jaime RC (2005). Efficacy of Several Serological Tests and Antigens for Diagnosis of Bovine Brucellosis in the Presence of False-Positive Serological Results Due to *Yersinia enterocolitica* O:9. *Clinical and Diagnostic Laboratory Immunology,* **12** (1): 141–151.

Nielsen K & Duncan JR, (1990). *Animal Brucellosis.* 1st edition. CRC Press, Boca Raton, Pp 1-442.

Nielsen K & Yu WL, (2010). Serological diagnosis of brucellosis. Section of Biological Medical Sciences, *The Macedonian Academy of Sciences and Arts (MASA)*, **31**(1): 65–89.

Nielsen K & Gall D (1998). Summary of field trials using the indirect and competitive enzyme immunoassays for detection of antibody to *Brucella abortus*. IAEA in Vienna, Austria.; 107-112, Pp 107-112.

Nielsen KH, Kelly L & Gall D, (1996). Comparison of Enzyme Immunoassays for the Diagnosis of Bovine Brucellosis. *Preventive Veterinary Medicine*, **26**(1): 7-22.

OIE (2008). (World Organization of Animal Health) Bovine Brucellosis, chapter 2.4.3., *Manual of Diagnostic tests and Vaccines for terrestrial animals (mammals, birds and bees)*, edited by World Organization of Animal Health, Pp 624 – 659.

Peniche CA, Martinez HDI, Franco ZJL, Barradas PF, Molina SB, Gutiérrez REJ,Williams JJ, Morales AF & Flores CR, (2008). Efficacy of S19 *Brucella abortus* strain in naturally infected bovine herds at the Mexican tropic. Brucellosis 2008 International Research Conference (Including the 61st Brucellosis Research Conference) Royal Holloway College, University of London, Pp 45.

Pepe MS & Janes H, (2007). Insights into latent class analysis of diagnostic test performance. *Biostatistics*, **8**(2): 474-84.

Peraza C, Valdes O, Fonseca N, Izquierdo L, Garcia M & Alvarez M (1997). Use of an indirect ELISA for *Brucella abortus* diagnosis in Cuba. Diagnosis and Epidemiology of Animal Diseases in Latin America, *Proceedings of the Final Research Co-ordination Meetings of FAO/IAEA/SIDA Co-ordinated Research Programmes*, Pp 89-94.

Philo M & Edwards WH, (2002). Brucellosis Diagnostics. *In: Brucellosis in Elk and Bison in the Greater Yellowstone Area, Jackson, Wyoming, (Kreeger TJ, editor)*. Wyoming Game and Fish Department, Pp 120-127.

Poester FP, Ramos ET & Thiesen SV (1997). Application of enzyme- linked immunosorbent assays for the diagnosis of bovine brucellosis in Rio Grande Do Sul, Brazil. Diagnosis and Epidemiology of Animal Diseases in Latin America Proceedings, of the Final Research Co-ordination Meetings of FAO/IAEA/SIDA Co-ordinated Research Programmes, Pp 61-68.

Refai M (2002). Incidence and control of brucellosis in the Near East region. *Veterinary Microbiology*, **90**(1-4): 81-110.

Rojas X & Alonso O (1996). ELISA for the diagnosis and epidemiology of *Brucella abortus* infection in cattle in Chile. Diagnosis and Epidemiology of Animal Diseases in Latin America, Proceedings of the Final Research Co-ordination Meetings of FAO/IAEA/SIDA Co-ordinated Research Programmes, Pp 77-82.

Samaha H, Al-Rowaily M, Khoudair RM, Ashour HM & (2008). Multicenter Study of Brucellosis in Egypt. *Emerging Infectious Disease,* **14** (12): 1916-1918.

Saravi MA, Wright PF, Gregoret RJ & Gall D (1995). Comparative performance of the enzyme linked immunosorbent assay (ELISA) and conventional assays in the diagnosis of bovine brucellosis in Argentina. *Veterinary Immunology and Immunopathology,* **47**(1-2): 93-99.

Stemshorn BW, Forbes LB, Eaglesome MD, Nielsen KH, Robertson FJ & Samagh BS (1985). A comparison of standard serological tests for the diagnosis of bovine brucellosis in Canada. *Canadian Journal of Comparative Medicine*, **49** (4): 391-394.

Thrusfield M (2003). Veterinary Epidemiology, Third edition. Black Well Publishing, Pp 313-330.

Vermunt KJ (1997). Lem: A general program for the analysis of cateriological data. Department of methodology and statistics, Tilburg University, Netherlands, Pp 1-101.