ejpmr, 2023, 10(5), 185-189

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SEROPREVALENCE OF *BRUCELLA ABORTUS* IN QALYUBIA GOVERNORATE, EGYPT

Ahmed Mohamed Fathi, Dalia Talaat Hassan and Madiha Salah Ibrahim

Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University, Egypt.

***Corresponding Author: Ahmed Mohamed Fathi**

Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University, Egypt.

Article Received on 24/02/2023 **Article Revised on 14/03/2023** **Article Accepted on 04/04/2023**

ABSTRACT

Brucellosis is considered one of the most common worldwide infections with heavy economic losses in animals due to abortion, reduction of milk, mastitis, retained placenta and reduced fertility. It is considered a serious human health hazard where infection can be mainly through drinking of unpasteurized milk and milk product, raw meat and contact with infected animals. The present study aimed to detect *Brucella abortus* infection among cattle and in contact persons in Qalyubia governorate, Egypt. Fifty milk samples were collected from suspected dairy cattle and 72 serum samples were collected from persons in contact with those cattle for serological analysis. *Brucella abortus* was detected by isolation and biochemical identification in 38 % of the milk samples, by MRT in 44% of the milk samples, while by RBT in 66.1% in human sera. Antimicrobial susceptibility analysis showed that all samples were resistant to all antimicrobials except gentamycin, tetracycline, streptomycin and chloramphenicol. In addition, Agar gel immunodiffusion test showed positive reaction.

KEYWORD: *Brucella abortus*, Rose Bengal test, Milk Ring test, Agar gel immunodiffusion.

INTRODUCTION

Brucellosis is one of the endemic diseases in Egypt affecting economy by damage of animals and human health **(Hussein et al., 2019)**. After Rabies, it is the 2nd common zoonotic disease in the world **(Abubakar et al., 2011)**. Brucellosis is caused by Brucella spp that is G-ve, aerobic, facultative intracellular, non-motile, non-spore forming and non-capsulated coccobacilli **(Godfroid et al., 2011; Kim et al., 2011)**. Brucella comprises 12 species, but zoonotic infection is mainly caused by 3 species; *B. mellitensis*, *B. abortus*, *B. suis* **(Seleem et al., 2010).**

Clinically it is a highly debilitating infection in which the case is presented with high undulant fever, anorexia, fatigue, headache and weight loss **(Corbel, 2006)**. When progressed to chronic disease, it shows severe joint illness and organ failure **(Quinn et al., 2002; Corbel et al., 2006)**. It is transmitted from domestic or wild animals through milk or close contact with the diseased animals **(Godfroid, 2017)**. The mode of transmission in human is ingestion of under cooked meat, raw milk or accidental inoculation of pathogen through wounds **(Akhvlediani et al., 2010)**. Abortion is a main clinical sign in animals **(Corbel et al., 2006; OIE, 2009)**. This study aimed to investigate the prevalence and antimicrobial susceptibility of *Brucella abortus* in milk samples. Further, to detect the prevalence of *Brucella abortus* antibodies in serum and milk samples.

MATERIAL AND METHODS

Serum samples

A total of 72 serum samples (male 29 and 43 female) were collected from laboratory of Benha Fever Hospital of the Ministry of Health in Qalyubia governorate, Egypt. Samples were collected and stored at -20°C until being examined serologically. Human consents from patients were collected before sample collection.

Milk samples

A total of 50 milk samples were collected from suspected diseased dairy cattle with history of mastitis during the period from January 2020 to August 2020. Midstream milk was collected from different quarters of the udder into sterile tubes. The samples were transferred on ice to the laboratory, Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University.

Rose Bengal test (RBT)

The serum and antigen of *Brucella abortus* **(Rose Bengal kit, BIOMED Co, Cairo, Egypt)** were brought at room temperature (22± 4°C) before testing, 30 µl of serum sample was placed on a white plate. Equal volume of antigen was placed next to the serum and thoroughly mixed using a sterile glass rod. The mixture was agitated gently for 4 minutes at 22±4°C on a rocker **(OIE, 2016)**.

Milk ring test (MRT)
The test was performed by adding 30 µl of Brucella antigen **(Hematoxylin-stained antigen manufactured by VSVRI, Abbassia, Cairo, Egypt)** to 1ml of whole milk that had been stored for at least 24hr at 4ºC **(Alton et al., 1988)**.

Isolation and identification of Brucella
Milk samples were centrifuged at 3500 rpm for 30 min. Milk cream from top and sediment were inoculated onto Brucella selective agar with selective Brucella supplement **(Oxoid) (Alton et al., 1988)**. Plates were incubated at 37ºC with 5% CO_2 and observed daily up to 14 days. Identification of the bacterial colonies in pure culture was performed by colony morphology, Gram's staining, catalase, oxidase, H_2S and urease tests **(Alton et al., 1975; Alton et al., 1988)**.

Antimicrobial susceptibility
Antimicrobial susceptibility was performed using the disc diffusion method **(Bauer et al., 1996).** The following antimicrobial discs were used: gentamicin (CN, 10 µg), tetracycline (TE, 30 µg), streptomycin (S, 10 µg), imipenem (IPM, 10 µg), chloramphenicol (C, 30 µg), rifampicin (RIF, 5 µg), erythromycin (E, 15 µg), and ciprofloxacin (CIP, 5 µg) **(**Oxoid, UK). The plates were examined for bacterial growth and the diameter of inhibition zones surrounding antibiotic disks were scored in millimeter (mm). The zone diameters were interpreted as resistant (R) or susceptible (S) according to the Clinical and Laboratory Standard Institute **(CLSI, 2019)**.

Agar gel immunodiffusion test
It was carried out according to **Ouchterlony and Nilsson, (1986) and Collins et al., (2011)**. Forty microliters of the antigen were pipetted into the center well and the tested serum was placed in the surrounding wells. Plates were read at 24 h and 48 h.

RESULTS

Bacterial isolation and identification
Gram-negative small coccobacilli were detected. Colonies appeared round, convex, 1-2 mm in diameter, with smooth margins, round edges, translucent and honey in color. Out of 50 milk samples, *Brucella abortus* was detected in 38% (19 of 50) of the samples only. Biochemical identification showed positive results for catalase, oxidase, H_2S production and urease tests.

Prevalence of *Brucella abortus*
Screening of the serum samples by RBT showed that 61.1% (44 of 72) of serum samples were positive for brucellosis. RBT titers were 1:80 and 1:160 in 81.8% (36 of 44) of the positive samples. While, titers between 1:320 and 1:1280 were found in 18.18% (8 of 44) of the positive samples. *Brucella abortus* screening by Milk ring test showed that 44% (22 of 50) of the milk samples were positive as shown in **Table.1**.

Table (1): Prevalence of Brucella abortus in human serum and cattle milk samples using Rose Bengal test (RBT) and milk ring test (MRT).

Species	Test	*Br. abortus*	
		Positive No. (%)	Negative No. (%)
Human (n=72)	Rose Bengal	44 (61.1 %)	28 (38.8 %)
Cattle (n= 50)	Milk Ring	22 (44%)	28 (56%)

Antimicrobial susceptibility
The antimicrobial susceptibility of isolated *Brucella abortus* was tested. *Brucella abortus* was sensitive to gentamycin, tetracycline, streptomycin and chloramphenicol as shown in Table.2.

Table (2): The antimicrobial resistance profiles of *B. abortus* isolated from cattle.

Antimicrobial Disc	R (%)	Susceptibility
Chloramphenicol	0	S
Ciprofloxacin	25	R
Erythromycin	87.5	R
Gentamicin	0	S
Imipenem	25	R
Rifampicin	37.5	R
Streptomycin	0	S
Tetracycline	0	S

R; resistant, S; sensitive.

Agar gel immunodiffusion
Serum samples showed lines of positive interaction between Brucella antigen and antibody in serum as shown in Figure. 1.

Figure 1: Agar gel immunodiffusion for *B. abortus* in serum samples.

DISCUSSION

In the present study, 50 milk samples and 72 blood sample were analyzed for the detection of *Brucella abortus*. The obtained results showed that brucella antibodies were present in cow milk based on screening by milk ring test (MRT) **(Table. 1)**. The prevalence was 44% (22 of 50) in milk sample and these results were confirmed by isolation of brucella from the same samples. This agrees with **Walid et al., (2016)** who showed 44.8% positivity of milk samples collected from Kafr El Sheikh. The very close results from 2016 to 2020 indicate that the control programs are still ineffective. MRT is considered as an ideal method for detecting infected cow and diagnosis of brucellosis in individual animals **(Noriello, 2004).** The MRT has demonstrated its usefulness to detect specific antibodies against *Brucella* spp. in bovine milk samples used at herd level **(Oie, 2009; Vanzini et al., 2001),** although its sensitivity may be impaired when it is used in large herds, and its use in small flocks is not recommended due to the expected low specificity **(Oie, 2009; OIE ,2009).** The lower incidence might be due to passive immunization of calves through colostrum of their infected dams **(Mohammed et al., 2011).** As in a study by **Gogoi et al., (2017),** he reported the prevalence of brucella antibodies in cattle to range from 0.00 to 18.75% with an overall prevalence of 10.53%. While, out of 72 human blood samples, 44 (61%) were positive which is nearly the same as **El – Ghitany et al., (2014)** whose study showed 62.1% positivity. With comparison, there is little difference because the source of infection is still out of control in addition to minimal awareness of the people. Although, another study by **El- Diasty et al., (2016)** stated that the prevalence of brucellosis among 295 individuals was 21% and they referred that for consumption of unpasteurized milk dairy products. Other studies by **Pappas et al., (2006) and Kelkay et al., (2017)** reported that approximately more than half a million new infected cases brucellosis were primarily an occupational disease affecting persons in close contact with infected animals. Also, **Farghaly et al., (2018)** found seropositivity for brucella among workers in farms to be 57.3% with RBT. Additionally, **Ramadan et al., (2019)** reported 22.22% seroprevalence among abattoir workers. The intracellular localization of *Brucella abortus* in the mononuclear phagocytic cells tends to resist the transportation of antibiotics through the cell membrane. Therefore, Brucella species hampers the efficiency of various antibiotics due to their prolonged development of resistance **(Hall and Manion, 1970).**

In addition, the antimicrobial test shows high sensitivity to gentamycin, tetracycline, streptomycin and chloramphenicol. All *B. Abortus* showed multidrug resistance against ciprofloxacin, erythromycin, imipenem and rifampicin. So, the findings in **Table** (**2**) agree with the results of **Barbosa et al., (2015).** The previous examination of *B. abortus* isolates of animal origin revealed the presence of resistance to rifampicin and ciprofloxacin in 25% and 37.5% of the isolates, respectively **(Khan et al., 2019).** The remarkable finding of the present study was the emergence of phenotypic antimicrobial resistance against erythromycin (87.5%). However, the increased use of these antimicrobials in Egypt in veterinary and human practices may be the cause of the emergence of this resistance **(WHO, 2013).** *B. abortus* isolates in this study were sensitive to chloramphenicol, gentamicin and tetracycline. These findings are comparable to previously published reports in Egypt, China, Qatar and Kazakhstan **(Abdel-Maksoud, et al., 2012; Deshmuk et al., 2015; Shevtsov, A et al., 2017; Liu et al., 2018).**

The agar gel immunodiffusion test (AGID) is based on precipitation of the antigen-antibody complex. This test is of low cost, it is easily performed and it has sensitivity levels that are comparable to complement fixation **(Myers et al., 1972; Ficapal et al., 1998; Xavier et al., 2011).** However, it has some disadvantages such as a marked decrease in sensitivity in chronic infections **(Gall and Nielsen, 2004; Xavier et al., 2011)** and high variability of the quality of commercially available antigens. Therefore, it is highly advisable to perform complementary diagnostic techniques such as PCR **(Costa et al., 2012).** Sensitivity of the agar gel immunodiffusion test varies from 50 to 92.7% and the specificity from 94.3 to 100% **(Marin et al., 1989; Hilbink et al., 1993; Ficapal et al., 1998; Estein et al., 2002; Gall and Nielsen, 2004; Xavier et al., 2011).** In the present study, Agar Gel immunodiffusion test showed Ag-Ab reaction line. Cattle infected with virulent, smooth strains of Brucella are therefore likely to develop antibodies to the agglutinogen. This is supported by observations of the very high titers of agglutinins present in the sera of cattle in the early and intermediate stages of *B. abortus* infection. However, the agglutinin levels decline in the chronic phase of the disease, probably because of shift in the pattern of immunoglobulin response **(Anderson et al., 1964; Rice et al., 1966).** The AGID test has demonstrated its usefulness to solve diagnostic interference problems in bovine and small ruminants **(Jones LM et al., 1980)**

CONCLUSION

Brucellosis is an endemic disease in Egypt, especially in Qalyubia that is considered an economic problem. The steps that should be taken to hinder spread of this disease are by slaughtering the infected and reservoir animals. Regarding zoonosis cutting the route by Boiling and/or pasteurization of milk and proper cooking of meat. The human in contact with animal have to be very aware about the disease and modes of transmission. The treatment must be based on culture and sensitivity to specific antibiotics and programmed surveillance studies.

Conflict of interest.

The authors declare no conflict of interest.

REFERENCES

1. Abubakar M, Mansoor M, Arshed MJ. Bovine brucellosis old and new concepts with282 Pakistan perspective. Pak Vet J., 2011; 32(2): 147-155.
2. Akhvlediani T, Clark DV, Chubabria G, Zenaishvili O, and Hepburn MJ, The changing pattern of human brucellosis :clinical manifestation, epidemiology, and treatment outcomes over three decades in Georgia BMC infection diseases, 2010; 10(1): 346.
3. Alton, G.G.; Jones, L.M.; Angus, R.D. and Verger, J.M. Bacteriological methods. Techniques for the Brucellosis Laboratory. Institute National de la Recherché Agronomique, Pairs, France, 1988; 13-61.
4. Barbosa Pauletti, R.; Reinato Stynen, A.P.; Pinto da Silva Mol, J.; Seles Dorneles, E.M.; Alves, T.M.; de SousaMoura Souto, M.; Minharro, S.; Heinemann, M.B.; Lage, A.P. Reduced susceptibility to rifampicin and resistance to multiple antimicrobial agents among Brucella abortus isolates from cattle in Brazil. https://doi.org/10.1371/journal., 2015.
5. Bauer AW, Kirby WMM, Sheriss JC, Turck M. Antibiotic susceptibility testing by standardized single method. Am J clin Path; 2(45): 493-496. Buffered plate antigen for use in a presumptive test for brucellosis. Dev. Biol. Stand, 1996; 56: 349-356.
6. Collins, M.T. Guest Editor. R.A. Smith Consulting Editor. Veterinary Clinics of North America – Food Animal Practice – Johne's disease. Volume 27, number 3. Saunders, Philadelphia, PA. 13 chapters, 14 authors, 2011; 143.
7. Corbel, M.J. Brucellosis in human and animals: World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organization for Animal Health. www.who.int/csr/resources/publications/Brucellosis. pdf, 2006.
8. Clinical and Laboratory Standard Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing, 29th ed.; CLSI: Wayne, PA, USA., 2019.
9. El - Ghitany EM, Omar SR, Abaza AM, Hassan EM, El-Wahab EWA. Trends in the Epidemiology of Brucellosis in a Highly Afflicted Region in Egypt: A 16 Year Experience (1997-2012). International journal of tropical disease and health, 2014; 4: 250-271.
10. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) Breakpoint Tables for Interpretation of Mics and Zone Diameters, Version 9.0. http://www.eucast.org, 2019.
11. Gall D, Nielsen K Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison Rev Sci Tech, 2004; 23: 989-1002.
12. Godfroid, J.; Scholz, H.C.; Barbier, T.; Nicolas, C.; Wattiau, P.; Fretin, D.; Whatmore, A. M.; Cloeckaert, A.; Blasco, J.M.; Moriyon, I.; Saegerman, C.; Muma, J.B.; Al Dahouk, S.; Neubauer, H. and Letesson, J.J.: Brucellosis at the animal /ecosystem /human interface at the beginning of the 21st century. Prev. Vet. Med., 2011; 13.
13. Godfroid J. Brucellosis in livestock and wildlife: zoonotic diseases without pandemic296 potential in need of innovative one health approaches. Arch Public Health, 2017; 297; 75(1): 34.
14. Hussein A.H., Mohamed R.H., Abdel- Ra, Ouf M.A., Abu- Elnag E.M.E., Mohamed S.R., Hussein E.A. and Axel Wehrend, Diagnosis of Brucellosis in recently aborted ewes using serological tests and polymerase chain reaction. J. Applied Sci., 2019; 19: 77-81.
15. Kim Km, Kim kw, Sung S, and kim H A genome - wide identification of genes potentially associated with host specificity of brucella species. The Journal of Microbiology, 2011; 49(5): 768-775.
16. OIE, Bovine Brucellosis: Diagnostic Technique Manual of Diagnostic Tests and Vaccines. 4th edition Paris, France: Office International des Epizootics, 2009; 1-37.
17. OIE, Terrestrial manual. Brucellosis Brucella abortus, B.melitensis and B. suis (infection with B. abortus, B. melitensis and B. suis), 2016.
18. Quinn, P.J. Markey, B.K., Carter, M.E., Donnelly, W.J. & Leonard, F.C. Veterinary Microbiology and Microbial Disease. Dublin: Blackwell Science Ltd, 2002; 162-167.
19. Seleem M, Boyle SM, and Sriranganthan N. Brucellosis: a re-emerging zoonosis. Vet Microbiol, 2010; 140: 392-398.
20. Walid E, Yamen H, Nour H. Abdel-Hamid, Essam M. Brucellosis at the Human-Animal Interface in Kafr elsheikh Governorate, Egypt, 2016.
21. World Health Organization (WHO) Regional Office for the Eastern Mediterranean. Report on the Consultative Meeting on Antimicrobial Resistance for Countries in the Eastern Mediterranean Region: From Policies to Action; World Health Organization, Regional Office for the Eastern Mediterranean: Sharm el Sheikh, Egypt, 2013; 270.
22. Khan A.U., Shell W.S., Melzer F., Sayour A.E., Ramadan E.S., Elschner M.C., Moawad A.A., Roesler U., Neubauer H., El-Adawy H. Identification, genotyping and antimicrobial susceptibility testing of Brucella spp. isolated from livestock in Egypt. Microorganisms, 2019; 7. doi: 10.3390/microorganisms7120603.
23. Anderson, R. K., Jenness, R., Brumfield, Helene P., Gough, Patricia Brucella-agglutinating antibodies: relation of mercaptoethanol stability to complement fixation. Science, N. Y., 1964; 143: 1334.
24. Costa, E.A.; Sant'ana, F.M.; Carvalho, C.J.S.; Moustacas, V.S.; Silva, S.M.M.S.; Paixão, T.A.; Santos, R.L: Diagnosis of Brucella ovis infection by serology and PCR in urine samples from naturally infected rams in the State of Piauí. Arquivo. Brasileiro de Medicina Veterinária e. Zootecnia., 2012; 64(3): 751-754.
25. Deshmukh, A.; Hagen, F.; Sharabasi, O.A.; Abraham, M.; Wilson, G.; Doiphode, S.;

Maslamani, M.A.; Meis, J.F. In vitro antimicrobial susceptibility testing of human Brucella melitensis isolates from Qatar between 2014–2015. BMC Microbiol, 2015; 15: 5.
26. El –Diasty, M.M.; Ahmed, H.A.; Sayour, A.E.; El Hofy, F.I.; Tahoun, A.B. and Shafik, S.M. Seroprevalence of Brucella spp. In cattle, Molecular characterization in milk, and the Analysis of Associated Risk Factors with Seroprevalence in human, Egypt. Vector Borne Zoonotic Dis., 2016; 16(12): 758-764.
27. Estein, S.M.; Baldi, P.C.; Bowden, R.A. Comparison of serological tests based on outer membrane or internal antigens for detecting antibodies to Brucella ovis in infected flocks. Journal of Veterinary Diagnostic Investigation, 2002; 14: 407-411.
28. Farghaly, R.M.; Amer, S.A.; Fahim, A.E.; Kishk, R.M. and Abbas, M.F. predictors of brucellosis seropositivity among exposed workers. Egyptian Journal of Occupational Medicine, 2018; 42(2): 209-226.
29. Ficapal, A.; Jordana, J.; Blasco, J. M.; Moriyón, I. Diagnosis and epidemiology of Brucella ovis infection in rams. Small Ruminant Research, 1998; 29: 13-19.
30. Gall, D.; Nielsen, K. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. Revue Scientifique et Technique, 2004; 23(3): 989-1002.
31. Gogoi, S.B., Hussain, P., Ch. Sarma, P., Barua, A.G., Mahato, G., Bora, D.P., Konch P. Gogoi, P. Prevalence of Bovine Brucellosis In Assam, India. J. Entomol. Zoology Studies, 2017; 5(4): 179-185.
32. Hall Wh, Manion Re. In Vitro Susceptibility of Brucella to Various Antibiotics Appl Microbiol, 1970; 20(4): 600-604.
33. Hilbink, F.; Wright, M.; Ross, G. Use of the Double Immune gel diffusion test and the enzyme-linked immunosorbent assay to distinguish false from true reactors in the complement fixation test for Brucella ovis. New Zealand Veterinary Journal, 1993; 41: 111- 115.
34. Jones LM, Berman DT, Moreno E, Deyoe BL, Gilsdorf MJ, Huber JD, and Nicoletti P. Evaluation of a radial immunodiffusion test with polysaccharide B antigen for diagnosis of bovine brucellosis. Journal of Clinical Microbiology, 1980; 12(6): 753-760.
35. Kelkay, M. Z., Gugsa, G., Hagos, Y., & Taddelle, H. Sero-prevalence and associated riskfactors for Brucella sero-positivity among small ruminants in Tselemti districts, Northern Ethiopia. Journal of Veterinary Medicine and Animal Health, 2017; 9(11): 320-326.
36. Liu, Z.G.; Di, D.D.; Wang, M.; Liu, R.H.; Zhao, H.Y.; Piao, D.R.; Zhao, Z.Z.; Hao, Y.Q.; Du, Y.N.; Jiang, H.; et al. In vitro antimicrobial susceptibility testing of human Brucella melitensis isolates from Ulanqab of inner Mongolia, China. BMC Infect. Dis., 2018; 18(6).
37. Marin, C.M.; Bagues, M.P.J.; Blasco, J.M. Comparison of three serological tests for Brucella ovis infection of rams using different antigenic extracts. Veterinary Record, 2019; 125: 504-508, 1989.
38. Mohammed F.U., Ibrahim S., Ajogi I., Olaniyi B.J.O. Prevalence of bovine Brucellosis And Risk Factors Assessment In Cattle Herds In Jigawa State. Isrn Vet. Sci., 2011.
39. Myers, D.M.; Jones, L.M.; Varela-Diaz, V.M. Studies of antigens for complement fixation and gel diffusion tests in the diagnosis of infections caused by Brucella ovis and other Brucella. Applied Microbiology, 1972; 23: 894-902.
40. Nielsen K. Diagnosis of brucellosis by serology. Veterinary Microbiology, 2002; 90(1-4): 447-459.
41. Noriello, S. Laboratory-acquired brucellosis. Emerg Infect Dis., 2004; 10: 1848-1850.
42. OIE. Caprine and Ovine Brucellosis (excluding Brucella ovis). Manual of diagnostic tests and vaccines for terrestrial animals, 2009; 2.7.2.
43. OIE. Bovine Brucellosis. Manual of diagnostic tests and vaccines for terrestrial animals, 2009; 2.4.3.
44. Ouchterlony., O. and Nilson, L.A., Immunodiffusion and immune electrophoresis. In Handbook of Experimented immunology ed. Weir, D.M Oxford and Edinburgh: Blackwell Scientific Publications, 1986; 1: 32-50.
45. Pappas, G., Photini, P., Nikolaos, A., Leonidas, C. The new global map of human brucellosis, 2006; 6(2): 91-99.
46. Ramadan, E.S., Nassar, N.R., Ibrahim, I.G. and Zayed A.F. Epidemiological and zoonotic surveillance of brucellosis in Beni-Suef governorate. AJVS, 2019; 61(1): 33-31.
47. Rice, C. E., Tailyour, J., And Cochrane, D. Shevtsov, A.; Syzdykov, M.; Kuznetsov, A.; Shustov, A.; Shevtsova, E.; Berdimuratova, K.; Mukanov, K.; Ramankulov, Y Antimicrobial susceptibility of Brucella melitensis in Kazakhstan. Antimicrob. Resist. Infect. Control., 1966; 6(5).
48. Vanzini VR, Aguirre NP, Valentini BS, Torioni de Echaide S, Lugaresi CI, Marchesino MD, and Nielsen K. Comparison of an indirect ELISA with the Brucella milk ring test for detection of antibodies to Brucella abortus in bulk milk samples. Veterinary Microbiology, 2001; 82(1): 55-60.
49. Xavier, M.N.; Sant'anna, F.M.; Silva, T.M.A.; Costa, E.A.; Moustacas, V. S.; Merlo, F.A.; Carvalho Júnior, C.A.; Dasso, M.G.; Mathias, L.A.; Gouveia, A.M.G.; Lage, A.P., Santos, R.L. A comparison of two agar gel immunodiffusion methods and a complement fixation test for serologic diagnosis of Brucella ovis infection in experimentally infected rams. Arquivo brasileiro de medicina veterinária e zootecnia, 2011; 63(4): 1016- 1021.