Benha Journal of Applied Sciences (BJAS) **Print: ISSN 2356–9751**
Vol. (1) Issue(2) June. (2016), (67-73) **Online: ISSN 2356–976x**
http:// bjas.bu.edu.eg

Heterologous Versus Homologous Antigens in the Serological Diagnosis of Small Ruminant Brucellosis

C.M.Roushdy[1], A.M.Selim[2], F.K..Hamouda[2], E.M.El-bauomy[1]
[1] Brucellosis Dept., Animal Health Research Institute, Dokki, Giza, Egypt
[2]Animal Medicine Dept., faculty of Veterinary Medicine, Benha Univ, Benha, Egypt
E-Mail: faisal.hamouda@fvtm.bu.edu.eg

Abstract

To elucidate controversial issue about variances between heterologous (B. abortus) and its counterpart homologous (B. melitensis) antigens used in serological examination of brucellosis in sheep and goats, sera of 250 sheep and 160 goats from seven Egyptian governorates were examined using both antigens in microplate serum agglutination (MAT) and complement fixation tests (CFT). The usefulness of melitensis antigen for diagnosis of small ruminant brucellosis was evaluated using a panel of selected immunoassays (BAPA, modified RB 8%, RB 3%, MAT and CFT with both abortus and melitensis antigens). The diagnostic sensitivity (D-se) and diagnostic specificity (D-sp) of such immunoassays were estimated. The significance and interaction of animal species, test categories as factors affecting results were assessed and results discussed. Kappa agreement between both antigens was substantial. No significant differences ($p > 0.05$) between groups of sheep and goats in MAT were found. Contrariwise, CFT showed significant difference ($p < 0.05$) between different groups of sheep and goats except between (sheep CFT melitensis and goat CFT abortus).

Keywords: Heterologous B.abortus antigen, Homologous B. melitensis antigen, CFT, MAT, Small ruminants.

1. Introduction

Brucellosis is wide-spread zoonotic infection in the developing as well as developed countries **[21]** It poses a major threat to human health and animal agriculture [2 Goats are the classic natural host of B. melitensis and together with sheep, are the preferred hosts [13]. At present, eleven species of Brucella are recognized [42] including three species that have been reported in Egypt [27]. It has been almost 3 decades of implementation of control program, yet brucellosis remains endemic among ruminants and humans in Egypt [24].

A precise diagnosis of Brucella spp. infection is important for the control of the disease in animals [30, 37] Clinical diagnosis is based usually on the history of reproductive failures in livestock, but it is a presumptive diagnosis that must be confirmed by laboratorial methods[36]. There are about 30 different serological tests for the diagnosis of brucellosis[5,30,33]. There is no single test that can correctly identify all infected cases in a single examination. The diagnosis of brucellosis is usually performed by a combination of methods. It is striking that no specific serological tests for B. melitensis infection of sheep have been developed. Instead, it is widely expected that the serological tests used for B. abortus in cattle are also suitable for the diagnosis of B. melitensis infection in small ruminants[4,18,25,15]. What makes this problem worse, is the absence of International Standard anti-Brucella melitensis serum (ISaBmS) till 2011 [26] .As both species carry a smooth-type lipopolysaccharide (LPS) with O chain variations [35] . All Brucella species are genetically related to each other [40]. Seeking for a better immunoassay in both sheep and goats is the major goal. Considering CFT, the classic gold standard and the reference test recommended by the [33] this study compares the performance of both B. abortus and B. melitensis antigens for the diagnosis of brucellosis in small ruminants using MAT and CFT tests to provide information for suitable tests in every species.

2. Material and methods

2.1 Samples

A total of 410 serum samples (250 sheep and 160 goat) were selected from the serum collection received for routine testing by the Department of Brucellosis Research, Animal Health Research Institute, from seven Egyptian governorates namely Alexandria, Dakahlia, Gharbyia, Minufyia, Sharquia, Beni-Suef and Luxor.

2.2 Serological examination

Buffered acidified plate antigen (BAPA), modified Rose Bengal plate (m RB 8%) and Rose Bengal (RB 3%) tests were used. Antigens for these immunoassays were obtained from the National Veterinary Services Laboratories (NVSL), Ames, Iowa, USA.

Buffered acidified plate antigen was performed according to [6] , m RB 8% according to [8] whereas RB 3% was done according to [28] .

Microplate serum agglutination (MAT) and complement fixation tests (CFT) were performed using B. abortus antigen obtained from the APHA Scientific (formally AHVLA Scientific) and B. melitensis antigen that was from the (NVSL, Ames, Iowa, USA).

The procedure of MAT was performed as described by [9] with a modification considering shaking incubation at 37°C for 90 minutes, centrifugation at 300 g for one minute then final reading [23] with addition of safranin as performed by [7]The used antigen was standardized to give

50% agglutination at 1/650 of the national Egyptian serum equivalent to the OIEISS international serum. A titer corresponding to 80 IU or more was considered positive, while a titer corresponding to 40-80 IU was considered suspicious and less than 40 IU interpreted as negative.

CFT was applied according to [5] and results of CFT were converted to ICFTU/ml and interpreted as positive at $\geq$ 20 ICFTU/ml.

2.3 Statistical analysis

By using SPSS® Statistics, Version 20, IBM®. The data were statistically tested for the normal distribution using SPSS. Unfortunately, the assumption was violated (right Skewed) as indicated by SPSS Shapiro-Wilk test at value of $p < 0.05$. Titers of MAT and CFT were converted to IU /ml of serum then data were transformed to follow normal distribution. One-way ANOVA with post hoc using Games-Howell test were used to study the statistical significant between the means of serological tests categories and their effect on serological test results. Determination of Kappa agreement (κ) between both antigens in the both tests MAT and CFT. In addition to measuring diagnostic sensitivity and specificity for each test considering the CFT with abortus antigen as the gold standard.

3. Results

A total 410 serum samples from sheep and goats had history of abortion were examined serologically to detect Brucella antibodies using different serological tests. The true positive results were 243 and 130 out of 250 and 160 serum samples in sheep and goats, respectively regarding to CFT a as the gold standard test. The obtained results showed higher percentage of true positives 242 in BAPA test, 241 in both m RB 8% and RB 3% tests, 239 in CFT m and nearly similar obtained results in MAT a and MAT m were (234) and (233) in sheep out of 243 serum samples as shown in Table (1) Unlike sheep, goats' results elucidated different percentages of true positives being in descending order BAPA (129), m RB 8% (129), RB 3% (128), MAT a (124), MAT m (121), and CFT m (108) respectively out of 130 serum samples Table (1) . The Diagnostic sensitivity (D-se) of CFT m was the highest all over applied immunoassays scored 100% on the expanse of specificity that recorded 83.1% in goats. Regarding to D-se, that was nearly similar in MAT m (96.3%) and MAT a (95.8%) in sheep but in goats, MAT a achieved little higher rate 95.3% than 93% in MAT m Table (2) . As a result of high agreement between the CFT m and the gold standard (CFT a), the recorded diagnostic specificity (D-sp) of CFT m was the highest among other applied immunoassays (98.3%) in sheep while both MAT a and MAT m had the same D-sp (71.4%) in sheep and (60%) in goats Table (2) Kappa test revealed substantial agreement between homologous and heterologous antigens applied in MAT and CFT as showed in table (3) .

Results of one-way ANOVA test and multiple comparisons using Games-Howell post hoc test in both CFT and MAT were shown in Tables (4,5) illustrated that there was significant difference between groups of the test (sheep CFT a, sheep CFT m, goats CFT a, goats CFT m) at the value of $p < 0.05$ except in interaction within one group (sheep CFT m and goats CFT a) that showed no significant difference at $p > 0.05$. Whilst within groups in MAT (sheep MAT a, sheep MAT m, goats MAT a, goats MAT m) the p value close to be non-significant at $p = 0.046$ that proved by post hoc multiple comparisons that showed no significant difference between groups of MAT at $p > 0.05$.

4. Discussion

After more than a century, no major country has been able to eradicate brucellosis following its widespread establishment [29] . Diagnosis of brucellosis in any species is not a trivial matter and the serological investigation is the mainstay of diagnosis relatively fast, non-hazardous, cheap, available and more sensitive and therefore preferred in routine clinical practice [36] The highest true positives in sheep and goats were recorded in BAPA which is an excellent qualitative presumptive test to start with in order to exclude negative cases from supplementary testing. The results were in agreement with [19] and [1] . As antigen acidification (3.70± 0.03) prevents IgM binding, favoring agglutination with IgG, and thus increasing the specificity for detection of Brucella species infection [36].

Both versions of Rose Bengal (m RB 8% and RB 3[1]%) detected high proportion of true positives which comes in accordance with [28,14] who described the 3% RB to be an appropriate screening test. The recommended modification (m RB 8%) increases sensitivity of RB and minimizes the discrepancies between RB and CFT results [8] that agree with results in the research. Regarding MAT, it was developed as a simpler and more efficient test than the tube serum agglutination (SAT) [7] These tests depend on B. abortus antigen [32] .The present study compares between using of B. abortus and B. melitensis antigen in MAT. The obtained true positive results were nearly similar in sheep and goats. The new technique of MAT used

in this investigation [23] shows that hasten time of incubation with shaking (90 minutes) followed by centrifugation at 300 g for 1 minute leads to fast reading of results that was close to that of overnight incubation although it requires good practice to get correct reading. Safranin-O stain provided contrast to agglutination reaction, and also the dye improved visualization and allowed clearer positive/ negative demarcation [16] . Previous studies about sensitivity of SAT varies from 29.1 to 100% and specificity from 99.2 to 100 [15,36,19] recorded the mean of sensitivities of SAT (75.9%), CFT (89.0%).

The CFT is considered to be the most effective test for diagnosing brucellosis in small ruminants [17] and the classic gold standard and the reference test recommended by the OIE[33] . As regards, CFT m differs greatly in both species recording 239 (97.2%) in sheep and 108 (81.25%) in goats that clarify dissimilarity against B. melitensis antigen in both species.

The prior studies of heterologous and homologous antigens in SAT by [39] agreed with [5] who recorded higher titers by using homologous antigen in SAT but these findings contrast what obtained by [22] who found higher titers by using heterologous[2] antigen. While [41] compared heterologous antigen S99 with the homologous M antigen in CFT and they declared no significant differences were detected among the various used antigens (B. abortus S99, B. melitensis M1, M2 and M3 antigens) either in terms of sensitivity or antibody kinetics; which support finding of the research.

Concerning D-se and D-sp of MAT using dual antigens in (table, 2) revealed that D-se were nearly similar in sheep (95.8% A / 96.3% M) and bit discrepancy in results of goats (95.3% A/ 93% M). Meanwhile, by using both antigens same recorded D-sp in sheep (71.4%) and in goats (60%). [3,41] reported similar sensitivities for homologous and heterologous antigens.

MAT is performed at a near neutral pH, which makes it more efficient in detecting IgM antibody. Hence, it is best used to detect acute infections. It is less effective for IgG, resulting in low assay specificity[11,31] . Due to this fact, the MAT, despite being sensitive, is generally not used as a single test, but rather it is used in combination with other tests. Other short comings of the test include false positive and false negative results[36] . Excess of antibodies resulting in false negative reaction due to prozone effect [2] the test wasn't recommended[33] . Regarding CFT m in sheep and goats D-se and D-sp were the higher among all over immunoassays due to high agreement between the CFT m and the gold standard CFT a. Regardless of the antigen, the levels of antibodies were lower in goats than in sheep [3] .

The kappa (κ) agreement in sheep and goats between both examined antigens in MAT and CFT were interpreted as substantial. The results agree with [41] who declared no significant differences were detected among the various antigens used (B. abortus S99, B. melitensis M1, M2 and M3 antigens) in CFT either in terms of sensitivity or antibody kinetics. The homologous antigens did not give a more sensitive result than that obtained using the standard antigen (S99) for detecting B. melitensis infections in sheep. Furthermore, they do not appear capable of detecting infections earlier, thus contradicting the findings of earlier authors[5,12,10] . There was significant difference between groups in MAT at $p < 0.05$ in ANOVA (table, 4). The actual difference in mean scores between groups has small effect 0.012. In contrary the results of post-hoc (table, 5) comparison between each two groups, showed no significant difference at $p > 0.05$. As titers of each group were fluctuated in both antigens and species and due to violations of the ANOVA assumptions that push me to choose the result of post-hoc rather than ANOVA in the final conclusion of significance difference. Finally, there was no significant difference between heterologous and homologous antigens in MAT. That may be explained by studies of [34] who have found such high homology within the genus and have proposed that all Brucella were actually one species. B. abortus and B. melitensis were the most closely related species.

Otherwise ANOVA in CFT at first glance reflected conspicuous significant differences between groups $p < 0.05$ and confirmed by post-hoc results that revealed all pairwise comparison attain significant differences except between sheep CFT m and goat CFT a. The actual difference in mean scores between groups was medium effect 0.09. An explanation of this result is what reported by[26] , at least for the moment, the CFT will continue to be standardized by the Second International standard for B. abortus antiserum (OIEISS) alone because it was impossible to set criteria for the complement fixation test even by using International Standard anti-Brucella melitensis Serum (ISaBmS). As there are difficulties in the harmonization of this technically demanding test.

Table (1) Results of different immunoassays applied on examined sheep and goats in relation to CFT with abortus antigen

CFT with abortus antigen			BAPA		modified RBT 8%		RBT 3%		MAT a		MAT m		CFT m	
			+	-	+	-	+	-	+	-	+	-	+	-
Sheep	+	243	242	1	241	2	241	1	233	10	234	9	239	1
	-	7	2	5	2	5	2	6	2	5	2	5	4	6
Goats	+	130	129	1	129	1	128	2	124	6	121	9	108	22
	-	30	10	20	12	18	12	18	12	18	12	18	0	30
Total animals	+	373	371	2	370	3	369	13	357	16	355	18	347	23
	-	37	12	25	14	23	4	24	14	23	14	23	4	36

MAT a: Micro-agglutination serumagglutination test with B. abortus antigen
MAT m: Micro-agglutination serum agglutination test with B. melitensis antigen
CFT m: Complement fixation test with B. melitensis antigen

Table (2) Estimation of Diagnostic sensitivity/ specificity of MAT and CFT m tests using homologous and heterologous antigens

Species	True positives/ true negatives	Diagnostic sensitivity/ specificity	MATa	MAT m	CFT m
sheep	243	Sensitivity%	95.8	96.3	85.7
	7	Specificity%	71.4	71.4	98.4
Goats	130	Sensitivity%	95.3	93	100
	30	Specificity%	60	60	83.1

Table (3) Results of observed agreement in both heterologous and homologous antigens in MAT and CFT with Kappa values

	CFT a versus CFT m				MAT a versus MAT m				
	Both tests positive	Both tests negative	Dis-agreement	Kappa Agreement (κ)	Both tests positive	Both tests suspicious	Both tests negative	Dis-agreement	Kappa Agreement (κ)
Sheep (250)	239	6	5	0.696 ± 0.129	193	19	10	28	0.641±0.059
	95.6%	2.4%	2%		77.2 %	7.6 %	4 %	11.2%	
Goats (160)	108	30	22	0.648 ± 0.065	103	11	23	23	0.686±0.059
	67.5%	18.75%	13.75%		64.375 %	6.875 %	14.375 %	14.375%	
Overall agreement	0.693 ± 0.055 Substantial agreement				0.668 ± 0.042 Substantial agreement				

Table (4) Results of one-way ANOVA test in both CFT and MAT

	Anova in CFT				Anova in MAT		
	df	**F**	**Sig.p value**		**df**	**F**	**Sig.p value**
Between groups	3	21.046	0.000	Between groups	3	2.677	0.046
Within groups	636			Within groups	636		
Total	639			Total	639		

df: degree of freedom *: significant at the level of 0.05

Table (5) Multiple comparisons using Games-Howell post hoc test

Games-Howell post hoc			**Games-Howell post hoc**		
(I) CFT	**(J) CFT**	***Sig.(p value)**	**(I) MAT**	**(J) MAT**	***Sig. (p value)**
	s_CFT m	0.020		s_MAT m	0.994
s_CFT a	g_CFT a	0.004	s_MAT a	g_MAT a	0.628
	g_CFT m	0.000		g_MAT m	0.111
	s_CFT a	0.020		s_MAT a	0.994
s_CFT m	g_CFT a	0.774	s_MAT m	g_MAT a	0.462
	g_CFT m	0.000		g_MAT m	0.054
	s_CFT a	0.004		s_MAT a	0.628
g_CFT a	s_CFT m	0.774	g_MAT a	s_MAT m	0.462
	g CFT m	0.001		g_MAT m	0.784
	s_CFT a	0.000		s_MAT a	0.111
g_CFT m	s_CFT m	0.000	g_MAT m	s_MAT m	0.054
	g_CFT a	0.001		g_MAT a	0.784

s: sheep g: goats a: abortus antigen m: melitensis antigen

*: significant at the level of 0.05

5. Conclusion

There is no statistical difference between heterologous and homologous antigens in MAT ($p > 0.05$), while in CFT there was significant difference ($p < 0.05$) with medium effect between both antigens with exception between sheep CFT m and goat CFT a (show no significant difference). Both antigens were nearly similar at the level of specificity and sensitivity. A panel of immunoassays must be performed to improve diagnostic situation especially in sheep and goats to avoid false results.

References

[1] N.H. Abdel-Hamid, M.H. Ebeid, F.K.Arnaout, M.M Elgarhy, E.M Elbauomy, A.Hanaa, A.E. Sayour. Serological and bacteriological monitoring of ruminant brucellosis in seven governorates with control program follow-up in three cattle farms. Benha Veterinary Medical Journal,vol. 23 (2),pp.254-263, 2012.

[2] M. M.Afifi, U. M. Abdul-Raouf, E. M. El-Bayoumy, A. M. Montasser, and H. A. Mohamad. Isolation and Biotyping of Brucella melitensis from Upper Egypt. Journal of American Science,vol.7 (3),pp. 99. 653-659 ,2011.

[3] B. Alonso-Urmeneta, C,Marin V. Aragon, J.M.Blasco, , R.Diaz, , and I.Moriyon, Evaluation of lipopolysaccharides and polysaccharides of different epitopic structures in the indirect enzyme linked immunosorbent assay for diagnosis of brucellosis in small ruminants and cattle. Clin. Diagn. Lab. Immunol, vol.5,pp. 749-754, 1998.

[4] G.G. Alton,. Brucella melitensis, Animal brucellosis, CRC, Press Boston, PP.1887-1987, 1990.

[5] G.G.Alton, L.M.Jones, R.D. Angus, J.M. Verger. Techniques for the brucellosis laboratory. Institute National de la Recherché Agronomique (INRA), Paris, France. 1988.

[6] R.D.Angus, C.E. Barton. The production and evaluation of a buffered plate antigen for use in a presumptive test for brucellosis. Dev. Biol. Stand., vol.56,pp. 349-356,1984.

[7] M. Baum, O. Zamir, R.Bergman-Rios, E.Katz, Z. Beider, A. Cohen, M. Banai,. Comparative Evaluation of Micro-Agglutination Test and Serum Agglutination Test as Supplementary Diagnostic Methods for Brucellosis. J. Clin. Microbiol, vol. 33(8),pp. 2166-2170,1995.

[8] J. M. Blasco, B. Garin-Bastuji, C. Marin, G., Gerbier, J. M. Fanlo, P. Jimenez de Bagues, C. Cau. Efficacy of different rose bengal and complement fixation antigens for the diagnosis of Brucella melitensis in sheep and goats. Vet. Rec, vol.134,pp. 415-420, 1994.

[9] W.J.Brinley Morgan, D. J.MacKinnon, K.P.W.Gill, S. G.M.Gower, P.I.W. Norris. Brucellosis diagnosis - Standard laboratory techniques (2nd Edition). Ministry of Agriculture, Fisheries and Food, London, UK, 1981.

[10] J. Connell, , G. Dubray, . Standardization of Rose Bengal Plate test and Allergic Skin Test for Diagnosis of Brucella melitensis infection in sheep and goats. E.C.R.N. B. melitensis. Report 3. Commission of the European Communities Scientific Workshop, Brussels, pp. 20-2,1 1986.

[11] M.J. Corbel, Characterization of antibodies active in the Rose Bengal plate test for bovine brucellosis. Vet. Rec.vol. 90, pp.484-485, 1972.

[12] M.J. Corbel,. Comparison of Brucella abortus and B. melitensis antigens for the rose Rose Bengal plate test on sera from cattle infected with B. abortus Biovar--5. Vet. Rec. vol.117, pp. 385-386 ,1985.

[13] E. Díaz-Aparicio. Epidemiology of Brucellosis in Domestic Animals Caused by Brucella melitensis, Brucella suis and Brucella abortus. Rev. Sci. et Tech. (International Office of Epizootics),vol. 32(1), pp .53-60, 2013.

[14] E.Diaz-Aparicio, , J. M.Blasco, , F. Suarez,. Evaluation of the modified card test for diagnosis of brucellosis in goats "Prueba de tarjeta modificada para el diagnostico de la brucelosis caprina." Veterinaria México 30(4): 307-311, 1999.

[15 E.] Diaz-Aparicio, C. Marin, , Alonso-B.Urmeneta,. Evaluation of serological tests for diagnosis of B. melitensis infection of goats. J. Clin. Microbiol., vol.32(5), pp. 1159-1165, 1994.

[16] E.M Elbauomy, A.E Sayour, N.H. Abdel-Hamid, M.K. El-Kholi, N.A. Shalaby. Validation of different versions of microtiter plate agglutination for rapid quantitative serologic diagnosis of Brucella melitensis infection in ruminants. Animal Health Research Journal,vol. 2 (4), pp. 79-90, 2014.

[17] FAO/WHO. Sixth report of the expert committee on brucellosis. Technical report series 740, Geneva, Switzerland: FAO/WHO, 1986.

[18] R.Farina,. Current serological methods in Brucella melitensis diagnosis. .in: Plommet, M., Verger, J. M. (Eds), Brucella melitensis., Martinus Nijhoff Publ., Dordrecht,pp. 139-146, 1985.

[19] , D.Gall, K.Nielsen,. Serological diagnosis of bovine brucellosis: A review of test performance and cost comparison. Rev. Sci. Tech. Off. Int. Epiz. Vol.23(3), pp. 989-1002, 2004.

[21] J. Godfroid, A.Cloeckaert, J.P Liautard, S.Kohler, D.Fretin, K.Walravens, B.Garin-Bastuji, J.Letesson, J. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Veterinary Research,vol. 36(3), pp. 313-326, 2005.

[22] S.Herr, J.V.Lawrence, O.L. Brett, L.M. Ribeiro. A serological comparison of complement fixation reactions using Brucella abortus and Brucella melitensis antigens in B. abortus infected cattle Veterinary Research Institute. The Onderstepoort Journal of Veterinary Research,vol. 58(2), pp. 111-114, 1991.

[23] S.Herr, C.Williamson, R.E.Prigge, A.Van Wyk,. The relationship between the micro-titration serum agglutination and complement fixation tests in bovine brucellosis serology. Onderstepoort, J. Vet. Res.,vol. 53(4) ,pp. 199-200, 1986.

[24] G.J.Jennings, R.A. Hajjeh, F.Y.Girgis, , M.A.Fadeel, M.A.Maksoud, M.O.Wasfy, N. El Sayed, P.Srikantiah, S.P.Luby, K. Earhart, F.J. Mahoney,. Brucellosis as a cause of acute febrile illness in Egypt. Trans. R. Soc. Trop. Med. Hyg.,vol. 101(7), pp.707–-713, 2007.

[25] A.MacMillan,. Conventional Serological Tests. In: Animal brucellosis. (Nielsen, K. and Duncan, J.R., eds). CRC Press, Boca Raton, pp.153-197, 1990.

[26] J.McGiven, A.Taylor, L.Duncombe, R.Sayers, D. Albert, M.Banai, J.M., Blasco, S. Elena, D.Fretin, B.Garin-Bastuji, F.Melzer, P.M.Muñoz, K.Nielsen, A.Nicola, M.Scacchia, M.Tittarelli, I.Travassos Dias, K.Walravens, J.Stack. The first International Standard anti-Brucella melitensis Serum. Rev. Sci. Tech. Off. Int. Epiz., vol.30(3), pp. 809-819, 2011.

[27] A.M.Menshawy, I.H.Hosein, R.A. Azzam, M.Perez-Sancho, N.García, I.Martinez, L. Dominguez, T.Garcia-Seco, A.E.Sayour, J.Alvarez. Assessment of Genetic Diversity of Zoonotic Brucella spp. Recovered from Livestock in Egypt Using Multiple Locus VNTR Analysis. Faculty of Veterinary

Medicine, Beni-Suef University, Egypt. BioMed Research International Volume 2014, Article ID 353876, Hindawi Publishing Corporationhttp://dx.doi.org/10.1155/2014/353876, 2014.

[28] A.B Mikolon, I.A. Gardner, S.K. Hietala, Anda JHde, E.C.Pestana, S.G.Hennager, A.J Edmondson. Evaluation of North American antibody detection tests for diagnosis of brucellosis in goats. Journal of Clinical Microbiology, vol.36(6), pp. 1716-1722, 1998.

[29] P.A. Nicoletti. Short history of brucellosis. Vet. Microbiol.vol. 90, pp. 5-9, 2002.

[30] K. Nielsen. Diagnosis of brucellosis by serology. Veterinary Microbiology,vol. 90, pp. 447-459 , 2002.

[31] K.Nielsen, F.Heck, G .Wagner.,. Comparative assessment of antibody isotypes to Brucella abortus by primary and secondary binding assays. Prev. Vet. Med. Vol.2, pp. 197-204, 1984.

[32] K.Nielsen, Yu, W.L.. Serological diagnosis of brucellosis Sec. Biol. Med. Sci., MASA, XXXI, vol.1, pp. 65-89, 2010.

[33] OIE, (World Organization for Animal Health). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 2.7.2. Caprine & Ovine Brucellosis (excluding Brucella ovis). OIE, Paris, France, 2009.

[34] S.C.Olsen, M. V. Palme Advancement of knowledge of Brucella over the past 50 years. Veterinary Pathology. On line, First, published on June 30, 2014 as doi: 10.1177/0300985814540545, 2014..

[35] M. B.Perry, D.R. Bundle.Antigenic Relationships of the Lipopolysaccharides of Escherichia hermannii Strains with Those of Escherichia coli 0157:H7, Brucella melitensis, and Brucella abortus. Infection and Immunity, vol.58(5), pp.1391-1395, 1990.

[36] F.P Poester, K.Nielsen, L.E.Samartino, W.L. Yu, .Diagnosis of Brucellosis. Open Vet. Sci. J.vol. 4, pp. 46-60, 2010.

[37] F.P. Poester, L.E. Samartino, R.L. Santos,. Pathogenesis and pathobiology of brucellosis in livestock. Rev. Sci. Tech. Of. Int. Epiz., vol.32(1), pp. 105-115, 2013.

[39] T.F.Salem, S.M.El-Gibaly Y.A. Farag, A.A.El-Molla, A. H. El-Molla. Evaluation of some of the commonly used serological methods for diagnosis of brucellosis. Agricultural Research review, vol.62(5A) , pp. 305-313, 1985.

[40] H.C.Scholz, G.Vergnaud. Molecular characterization of Brucella species. Rev. Sci. Tech. Off. Int. Epiz.,vol. 32(1), pp. 149-162, 2013 .

[41] M.Tittarelli, A.Giovannini, A.Conte, M. Di.Ventura, D.Nannini, V. Caporale,. The Use of Homologous Antigen in the Serological Diagnosis of Brucellosis Caused by Brucella melitensis. Journal of Veterinary Medicine, Series B. vol.52(2), pp.75-81, 2005.

[42] A.M.Whatmore, N.Davison, A.Cloeckaert, S. Al Dahouk, M.S.Zygmunt, , S.D. Brew, L.L.Perrett, M.S.Koylass, G.Vergnaud, C.Quance, H.C.Scholz, Jr. Dick, E.J., Hubbard, , and G.Schlabritz- N.E. Loutsevitch,. Brucella papionis sp. nov., isolated from baboons (Papio spp.). Int. J. Syst. Evol. Microbiol.,vol. 64, pp. 4120-4128, 2014 .

Benha Journal of Applied Sciences (BJAS) **Print: ISSN 2356–9751**
Vol. (1) Issue(2) June. (2016), (67-73) **Online: ISSN 2356–976x**
http:// bjas.bu.edu.eg