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Detection of *Brucella melitensis* by AMOS-PCR Assay and Histopathological Findings in Tissue of Serologically Positive Buffalo-cows

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Abstract: Brucellosis is a highly infectious disease which is diagnosed by serological and microbiological methods. The objective of this study was to assess the availability of AMOS PCR assays as a potential diagnostic tool for the detection and differentiation of brucellosis in naturally infected buffalo cows under Egyptian condition. A total number of 37 brucella sero-positivite (RBPT) adult buffalo cows which were subjected to official obligatory slaughtering at Sharkia governorate, Egypt was used. The results of RBPT were compared with L-ELISA, PP-ELISA, BAPAT, TAT, RVT, bacterial isolation as well as AMOS-PCR assay for both the isolate and tissue samples. Histopathological and the immunohistochemistry were carried out on infected tissues to confirm the infection. ELISA, using periplasmic protein antigen (PP-ELISA) had the best combined sensitivity and specificity as a screening test and can replace the RBPT as it avoiding the serological cross-reactions. *Br. melitensis* biovar3 was isolated. The AMOS-PCR identified and differentiated the *brucella* Spp. from buffalo's tissue in one step and it was superior to the bacterial isolation. The most common histopathological findings of brucellosis in buffalo-cows were chronic endometritis and granulomatous mastitis. The immunohistochemistry showed presence of brucellae antigen in the tissue of mammary gland, supramammary L.N., spleen and uterus..To our knowledge, this is the first time to use the AMOS –PCR to identify and differentiate *Brucella* species from the tissues of obligatory slaughtered buffaloes. In conclusion RBPT must be replaced as an official screening test by PP-ELISA assay as a highly sensitive and specific screening test, followed by AMOS-PCR.

Key words: *Brucella melitensis* · Buffaloes · Serology · Pathogenesis · Immunohistochemistry · Amos-PCR

INTRODUCTION

Brucellosis is a widespread zoonotic disease which constituting a serious public health problem in the Mediterranean and Middle-East countries [1]. Also, this disease is responsible for great economic losses in cattle and buffalo farming..Currently, the diagnosis of brucellosis is based almost entirely on serological tests [2] , although, these tests have proved to be either too sensitive, giving false positive results, or too specific, giving false-negative results[3]. In addition, the presence of antibodies may indicate infection, vaccination or cross reaction with other gram-negative bacteria [4].

The gold standard based on the isolation of suspicious bacterial colonies from host followed by differentiation of *Brucella* species [5] has drawbacks; like the length of the process besides the tests need highly skilled personnel and the zoonotic nature of *Brucella* species. Finally the results are not always definitive [6].

PCR proved to be rapid, highly sensitive, very specific, inexpensive and easily adapted for high volume demands. The AMOS-PCR (from abortus-melitensis-ovis-suis) succeeded to identify and differentiate most *Brucella* species [7] based on the location of some copies of the repetitive element IS711 in the genome of the different species [8].

Immunohistochemical technique is used for direct detection of small number of brucella antigen in fresh formalin-fixed and paraffin embedded tissues from animals that revealed low titer of antibodies and may contain few numbers of brucella organisms [9].

The present study was carried out to detect Brucella *melitensis* infection in obligatory slaughtered buffaloes with the aid of recent biotechnological methods. Also,

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carrying out some histopathological and immunohistochemical investigations was another target.

MATERIALS AND METHODS

Animals: A total number of 37 adult buffalo cows, subjected to official obligatory slaughtering at Sharkia abattoirs, Egypt due to brucella sero-positivity (using Rose Bengal Plate Test) was used in this study.

Samples: Blood samples were collected and then serum samples were separated and preserved at -20°C. Tissue samples (Supramammary lymph nodes, mammary glands, spleen and uterus) were taken for bacterial isolation, Polymerase Chain Reaction (AMOS-PCR), as well as histopathological and immunohistochemical examinations according to Bancroft *et al.* [10].

Bacterial Strains: *Brucella* reference strains (*abortus* 544, *melitensis* 16M and *suis* 1330) were kindly supplied by the Central lab for vaccines and sera, Abbasia, Cairo. All bacteria were manipulated according to Halling and Zehr [11].

Serological Tests: Seroprevalence of brucellosis was investigated by ELISA using crude lipopolysaccharide (LPS) according to Tittarell *et al.* [12] and periplasmic protein (PP) antigens of *Br. Abortus* S19 according to Yifan *et al.* [13]. The optimum antigen concentration and serum dilution were determined according to Narayanan *et al.[14]*. ELISA reading equal to or higher than double folds reading of negative controls was considered positive [15].

Buffered Acidified Plate Antigen Test (BAPAT, [16], Rose Bengal Plate Test (RBPT, [17], Tube Agglutination Test (TAT, [18] and Rivanol Test (RVT, [18] were performed.

Bacteriological Examination: Tissue specimens were subjected to brucella isolation and identification according to Alton *et al. [18]*.

Polymerase Chain Reaction (AMOS-PCR)

AMOS-PCR on Reference Bacterial Strains: With a sterile inoculating loop, a small quantity of inoculum was suspended in 0.5 ml of 0.85% sterile saline. The cell suspension (2.5 ml) was added to 22.5 ml of the master mix consisting of 60 mM Tris-HCl (pH 9.0), 15 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 250 mM concentrations of each of the four deoxy-nucleoside triphosphates, 1 U of Taq polymerase and five-primer cocktail (0.2 mM each), as previously described[7]. The mixture was cycled 35 times through a regimen of 1.2 min at 95°C, 2.0 min at 55.5°C and 2.0 min at 72°C under the conditions previously described [19]. The PCR products were analyzed by electrophoresis through a 1.5% agarose gel, after which the gel was stained with ethidium bromide and photographed [7].

AMOS-PCR on Buffaloes Tissues: The frozen tissue samples from all the 37 obligatory slaughtered animals were thawed at room temperature and the extraction of the genomic DNA was done using QIAamp DNA Mini Kit (Qiagen, Cat. No. 51304) depending on Mini Spin Columns where up to 25mg of the tissue samples were used ,while 5 µl of target DNA per 50 µl reaction mixture was used in the AMOS-PCR as mentioned above.

Immunohistochemistry: Tissue specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin, sectioned at 5µm and fixed to positive charge slides, Deparaffinization , enzyme treatment, Endogenous peroxidase blockage, conjugation , substrate addition and evaluation with the light microscope to determine the presence and cellular location of positive immunostaining according to Haines and Chelack[20].

RESULTS

Serological Investigation: In this work, the highest incidence of positive reactors was given by ELISA using Crude lipopolysaccharide Antigen (L-ELISA) , BAPAT and RBPT followed by ELISA using Periplasmic Protein antigen (PP-ELISA) then, RVT and TAT as shown in Table 1.

Bacteriological Examination: Biochemical identification indicated that, *Br. melitensis* biovars 3 was isolated from the collected tissue samples in 33 out of 37 (89.1%) buffaloes.

Polymerase Chain Reaction (PCR): The AMOS-PCR method could differentiate the different *Brucella* reference strains (*B. abortus, melitensis, suis*) as shown in Fig. 1. In the same time this PCR method on the tissue samples was indicative of *Br. melitensis* infection in 35 out of the 37 (94.5%) seropositive buffaloes as shown in Fig. 2.

Histopathological Findings: Histopathological examination of the uterus showed chronic endometritis. Granulomatous form was observed in 8 cases, the

Table 1: Incidence of Brucella seropositivity among obligatory slaughtered Brucella seropositive buffaloes

Item	L-ELISA	PP-ELISA	BAPAT	RBPT	TAT	RVT
NO	37	35.0	37	37	25.0	29.0
%	100	94.5	100	100	67.5	78.3

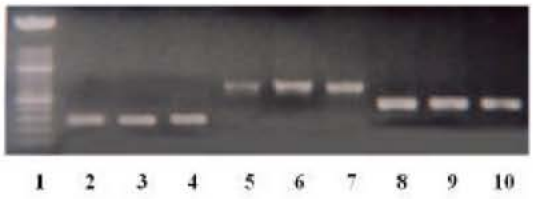

Fig. 1: AMOS- PCR for detection of Brucella Reference strains. Electrophoretic pttern of PCR product in 1.5% agarose gel stained with ethidium bromide Lane 1 : DNA ladder (100bp), Lanes 2,3,4 *B.suis* 1330 (285 bp), Lanes 5,6,7 *B.melitensis* 16M (731bp), Lanes 8,9,10 *B.abrtus* 544 (498bp)

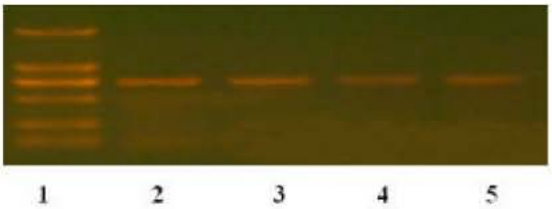

Fig. 2: PCR amplification of Brucella DNA from buffaloes tissue samples.
Lane 1: DNA ladder (100, 250, 500, 750, 1000, 20000 bp), Lane 2 : Positive control for *Br.melitensis* DNA (731bp), Lanes 3,4,5 : Tissue samples PCR product (731bp)

endometrium showed partial desquamation with lymphocytic infiltration. The granuloma formed of aggregations of phagocytic cells (Fig. 3A), with Perivascular and peri glandular mononuclear inflammatory cells infiltration associated with some necrotic glands. Proliferative *form of endometritis was seen in* 16 cases, the epithelial of the endometrium showed partial stratification, fibroblastic proliferation as well as mononuclear inflammatory cells aggregation in the stroma. Periglandular and perivascular mononuclear inflammatory cells aggregations were seen (Fig. 3B). Endometrial glands showed atrophy and narrowing of its lumen.

Supramammry lymph node showed hyperplastic lymohoid follicles which appeared large with wide germinal centers and highly activated lymphoblasts, focal and diffuse mononuclear cell aggregations in the medullary sinuses with extensive fibrous convective tissue proliferation (Fig. 3C).

Mammary gland showed *chronic mastitis,* Both granulomatous and proliferative mastitis were seen. There was a granulomatous structure in 21 cases, found in the glandular parenchyma, composed of central area of caseous necrosis surrounded with a zone of mononuclear inflammatory cells mainly macrophages and plasma cells (Fig. 3D). While proliferative mastitis was detected in 16 cases, characterized by interlober and interlobular fibrous connective tissue proliferation. Atrophy of the secretory acini with degeneration of the epithelium lining and narrow lumen, associated with infiltration of inflammatory cells mainly lymphocytes. Massive peri-acinal infiltration of mononuclear inflammatory cells mainly lymphocytes with cystic dilatation of some acini was clear, while other acini showed papillary hyperplasic projections toward the lumen.

Immunohistochemistry: In positive cases deposition of golden brown chromogen pigment at the site of antigen-antibody complex was seen in the cytoplasm of macrophages in the germinal center of lymphoid follicle in supramammary lymph node (Fig. 3E).

DISCUSSION

Diagnosis of brucellosis is complicated matter due to the variable incubation time and the absence of clinical signs other than abortion [1].

According to the Egyptian regulations, a test-and slaughter program had been instituted in Egypt for the control of brucellosis in cattle and buffaloes depending on the serological diagnosis using the RBPT. Accurate screening tests are important for the success of this control program. Therefore, here the RBPT was compared with the ELISA, BAPAT, TAT and RVT. These tests were chosen because they are easy to perform and they have been used in other countries for the diagnosis and eradication of brucellosis infection in livestock.

the present results for both ELISA using crude lipopoly-saccharide and BAPAT were identical. This finding proved the high sensitivity of both L-ELISA and BAPAT and enable them to replace the RBPT and came in agreement with the findings of Nielsen [21] and Gall and Nielsen [22].

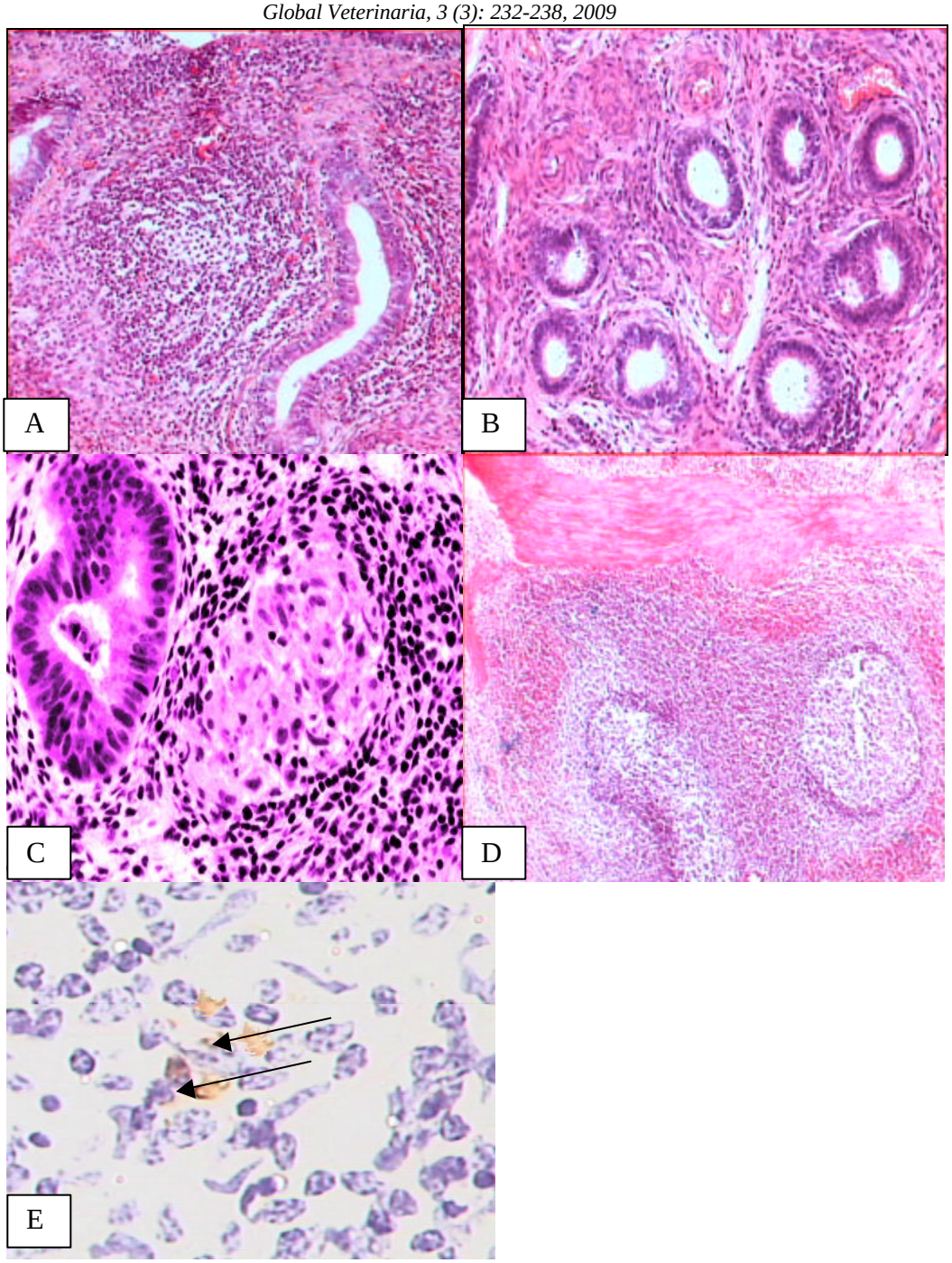

Fig. 3: Uterus of buffalo calf revealed seropositive reaction for brucella showing:

(A): Granulomatous reaction in the stratum compactum (H&E ; X100)

(B): Periglandular and perivascular mononuclear cells infiltration and fibroplastic proliferation (H&E;X100).

(C): Supramammary lymph node of buffalo showing follicular hyperplasia and extensive fibrous C.T. proliferation of the fibrous trabeculea (H&E; X40).

(D): Mammary gland of buffalo showing granulomatous structure. (H&E; X40).

(E): Supramammary lymph node revealed deposition of golden brown chromogen pigment at the site of antigen-antibody complex in the cytoplasm of macrophages

The low sensitivity of the PP-ELISA (94.5%) in comparison to the L-ELISA may be related to the difference in the binding of protein bands to ELISA polystyrene plates [23]. The failure of the PP-ELISA to identify all the RBPT positive reactors may be due to the relative high specificity of the PP-ELISA due to it eliminates the cross reactions with crude LPS antibodies of other gram negative bacteria as reported by Godfroid *et al.* [2]. This test was proved to be of the best combined sensitivity and specificity as it was the nearest in its results (94.5%) to the bacterial isolation (89.1%) and was identical in its result to the AMOS-PCR (94.5%).

The low sensitivity of the TAT (67.5%) could be due to this test can't detect the low titers as reported by Shalaby *et al.* [24] beside that the acidic pH of the RBPT enhances the agglutination of IgG1 immuno-globulin [25].

Using biochemical identification, *Brucella melitensis* biovars 3 was isolated from the collected tissue samples in 33 out of 37 (89.1%) obligatory slaughtered buffaloes. This finding came in agreement with that of Ghazi *et al.* [26]. The difference between the results of the RBPT and the bacterial isolation may be due to the RBPT false positive reactions; therefore it is necessary to use other tests to confirm reactor animals as indicated by Nielsen [21].

If the successful isolation of *Brucella* organisms is used as the gold standard method, incorrect Sp estimates might occur because of the miss-classification of some infected animals as uninfected. Another limitation of bacterial culture is that it necessitates lymph node biopsy or the collection of specimens at slaughter. Culling of animals as a method of diagnosis might not be appropriate or feasible under all economic and agricultural conditions [27].

The difference in results of AMOS-PCR (94.5%) and bacterial isolation (89.1%) may be due the presence of microbial contaminants in the samples and loss of viability of the organism before culturing or by the inhibition of some *Br. melitensis* strains in the selective medium [28].

Nucleic acid-based detection methods, such as PCR, are very promising tools for diagnostics. Primers derived from insertion sequence IS711 provides discrimination between the four *Brucella* species as reported by Casanas *et al.* [29]. Here in, it is the first time to use the AMOS-PCR assay for the detection and differentiation of *brucella* in buffalo's tissues.

In this study, PCR was shown to be a valuable tool for identification and differentiating the strains of *Brucella* in a single step even in buffalo's tissues. The conventional methods of identification require a minimum of 5 days to identify an isolate to *Brucella* species and biovar level. This can delay the movement of animals between different owners and have a negative impact on the owners' financial planning [30]. This study indicates that Brucellosis eradication program personnel could reliably use the *Brucella* AMOS-PCR to supplement other diagnostic and epidemiological data to release sale animals from quarantine before the conventional identification methods are completed.

The histopathological finding of the uterus in the present study showed by granulomatous endometritis characterized by fibroblastic cell proliferation, hyalinization of the tunica media and narrowing of the blood vessels lumen with Few glands surrounded by thick layers of C.T. and inflammatory cells agreed with the finding of Abd- Elrazik *et al.* [31].This indicated the chronicity of the condition and reflects the nature of the persisting infection [32].

It can be concluded that the most common type of endometritis associated with brucellosis in buffaloes was of the chronic type, this may be due that the expression of the pathological lesions of domestic animals is influenced by species and strain of Brucella, immune status of the host and route of exposure [32]. Also, it depends on the ability of *Brucella* to survive and persist intracellularly within professional and non professional phagocytic cells [33].

The histopathological finding of the supramammary lymph node this study showed as thickening of C.T. capsule and trabecullae as well as lymphoid depletion and came in accordance with Elmahdy *et al.*[34] who reported the deposition of fat in the lymph nodes draining area of fat necrosis.. In addition, lipids may occurred outside the cells when these cells suffer from necrosis leading to release of lipids into extracellular spaces, where pooling may make them visible [35].

In some of the present cases, the germinal centers were replaced by eosinophilic structureless mass of hyalinization. In this regard, it was reported that in the late stages of the infection, the lymph nodes draining the head, mammary glands and reproductive tract developed chronic granulomatous lymphadenitis which is usually associated with cortical and paracortical lymphoid depletion and germinal center expansion due to localization and replication of the microorganism in the macrophages and lymphocytes [36, 37].

On the light of the immunohistochemical results of the present work the entrance of *Brucellae* into the tissue of mammary gland could be explained as; brucella is probably carried from the blood stream into the mammary

gland inside phagocytic leukocytes which localized in mammary ducts and alveoli [38]. The degenerated phagocytes were often filled with intact *Brucellae* and extra cellular *Brucellae* was associated with ruptured phagocytes [36, 38].

In conclusion, the current results showed the necessity of replacing the RBPT as an official screening test with PP-ELISA assay as it is a highly sensitive and specific screening test, while AMOS-PCR and/ or immunohistochemistry can be applied in case of necessity on biopsy samples from mammary lymph tissues of the Egyptian buffalos. The most common histopathological findings on brucellosis in buffaloes were chronic endometritis and Granulomatous mastitis.

REFERENCES

1. Montagnaro, S., M. Longo, K. Mallardo, G. Pisanelli, L.De Martino, G. Fusco, L. Baldi, U. Pagnini and G. Iovane, 2008. Evaluation of a fluorescence polarization assay for the detection of serum antibodies to *Br. abortus* in water buffalo (*Bubalus bubalis*). Vet. Immunol. Immunopathol., 125: 135-142.
2. Godfroid, J., C., Saegerman, V. Wellemans, K. Walravens, J.J. Letesson, A. Tibor, A. McMillan, S. Spencer, M. Sanna, D. Bakker, R. Pouillot and B. Garin-Bastuji, 2002. How to substantiate eradication of bovine brucellosis when a specific serological reactions occur in the course of brucellosis testing. Vet. Microbiol., 90: 461-477.
3. Hamdy, M.E.R. and A.S. Amin, 2002. Detection of *Brucella* Species in the Milk of Infected Cattle, Sheep, Goats and Camels by PCR. Vet. J., 163: 299-305.
4. Diaz, R. and I. Moriyon, 1989. Laboratory techniques in the diagnosis of human brucellosis. In Brucellosis: Clinical and Laboratory Aspects. eds. E. J. Young and M. Journal of Corbel. Boca Raton, Fla, CRC Press, Inc., pp: 73-83.
5. Alton, G.G., L.M. Jones and D.E. Pietz, 1975. Laboratory techniques in brucellosis. Monograph Series, World Health Organization, Geneva, Switzerland.
6. Navarro, E., M.A. and Casao, J. Solera, 2004. Diagnosis of human brucellosis using PCR. Expert Review Molecular Diagnosis, 4: 115-123.
7. Bricker, B.J. and S.M. Halling, 1994. Differentiation of *Brucella abortus* bv. 1, 2 and 4, *Brucella melitensis, Brucella ovis* and *Brucella suis* bv. 1 by PCR. J. Clin. Microbiol., 32: 2660-2666.
8. Ocampo-Sosa, A.A., J. Aguero-Balbin and J.M. Garcia-Lobo, 2005. Development of a new PCR assay to identify *Br. abortus* bvs 5, 6 and 9 and the new subgroup 3b of biovar 3. Vet. Microbiol., 110: 41-51.
9. Essmail, M.E., I.G.A. Ibrahim and M.H. Yassen, 2002. Immuno-histochemical detection of *Brucella* antigens in formalin-fixed, paraffin-embedded tissues of Buffalos. J. Egyptian Vet. Medical Assoc., 62(5): 127-136.
10. Bancroft, J.D., A. Stenes and D.R. Turner, 1996. Theory and practice of histological technique, 4th Edn., Churchill Livingstone inc., New York.
11. Halling, S.M. and E.S. Zehr, 1990. Polymorphism in *Brucella* spp. due to highly repeated DNA. J. Bacteriol., 172: 6637-6640.
12. Tittarelli, M., M., Di Ventura, F. De Massis, M. Scacchia, A. Giovannini, D. Nannini and V. Caporale, 2005. The persistence of *Br. melitensis* in experimentally infected ewes through three reproductive cycles. J. Vet. Med., B52: 403-409.
13. Yifan, Z., J. Yang and C. Cheers, 1993. Cytokine response of T-cell subsets from *Brucella abortus*-infected mice to soluble bacteria proteins. Infectious Immunol., 61(7): 2841-2847.
14. Narayanan, P.R., G.S. Acharyulu and P.V. Krishnamurthy, A. Rauoof and S.P. Tripathy, 1983. Evaluation of ELISA as a diagnostic test in pulmonary tuberculosis. Indian J. Tuberculosis, 30: 29-32.
15. Bassiri, M., S. Ahmed, L. Giavendoni, L. Jones, J.T. Saliki, C. Mebus and T. Ilma, 1993. Immunonological response of mice and cattle to *baculovirus* expressed F and H proteins of *Rinderpest* virus, Lack of protection in the neutralizing antibody. J. Virol., 67: 1255-1261.
16. Anon, L., 1984. Supplemental test procedures for the diagnosis of brucellosis, Diagnostic reagent manual 65.E), National Veterinary laboratories, Ames, Iowa, USA.
17. Morgan, W.J.B., D.J. Mackinnon, K.P.W. Gill, S.G.M. Gower and P.I.W. Norris, 1978. Brucellosis Diagnostic Laboratory Techniques, 2nd Edn. Central Veterinary Laboratory, New Haw, Weybridge, pp: 11-53.
18. Alton, G.G., L.M. Jones, R.D. Angus, J.M. Verger, (Eds.), 1988. Tech-niques for the Brucellosis Laboratory. Institut National de la Recherche Agronomique, INRA, Paris.

19. Ewalt, D. and B. J.Bricker, 2000. Validation of the Abbreviated *Brucella* AMOS PCR as a Rapid Screening Method for Differentiation of *Brucella abortus* Field Strain Isolates and the Vaccine Strains, 19 and RB51. J. Clin. Microbiol., pp: 3085-3086.
20. Haines, D.M. and B.J. Chelack, 1991. Technical considerations for developing enzyeme Immunohisto-chemical staining procedures on formalin-fixed, paraffine embedded tissues for diagnostic pathology. J. Vet. Diagnostic Investigation, 3: 101-112.
21. Nielsen, K., 2002. Diagnosis of brucellosis by serology. Vet. Microbiol., 90: 447-459.
22. Gall, D. and K. Nielsen, 2004. Serological diagnosis of bovine brucellosis: A review of test performance and cost comparison. Rev. Sci. Technol., 23 (3): 989-1002.
23. hringi, B.N., S.S. Soni and K.N. Sharma, 2002. Seroactivity of *Br. abortus* antigens for the diagnosis of brucellosis. Indian Vet. J., 74: 432-434.
24. shalaby, M.N.H., H.M. Ghobashy, E.M. EL-Bauomy and M.A. Salh, Waffa, 2003. Prevalence of brucellosis among animal species in some governorates in Egypt. 7th Sci. Cong., Egyptian Society for cattle Diseases, 7-9 Dec., Assuit, Egypt, pp: 271-282.
25. Abdel-Kader, H.A., 1996. Serological study on caprine brucellosis in Assuit governorate. Assiut Vet. Med. J., 36: 9-16.
26. Ghazi, Y.A., K.A. Abd El-Razik and M.B. Kadry, 2006. Evaluation of *Brucella* diagnostic techniques in the Egyptian buffaloes. Proc. 3rd Inter Conf.Vet. Res. Div., NRC, Cairo, Egypt, pp: 23-34.
27. Fosgate, G.T., A.A. Adesiyun, D.W. Hird and S.K. Hietala, 2006. Likelihood ratio estimation without a gold standard: A case study evaluating a brucellosis c-ELISA in cattle and water buffalo of Trinidad. Preventive Vet. Med., 75: 189-205.
28. Refai, M.K., 2003. Brucellosis in animals and man in Egypt. Egyptian Vet. Sci., 37: 11-31.
29. Casanas, M.C., M.I. Queipo-Ortu˜no, A. Rodriguez-Torres, A. Orduna, J.D. Colmenero and P. Morata, 2001. Specificity of a PCR assay of a target sequence on the 31-kilodalton *Brucella* antigen DNA used to diag-nose human brucellosis. European J. Clin. Microbiol. Infectious Diseases, 20: 127-131.
30. Gupta, V.K., K. Verma, Deepak P.K. Rout, S.V. Singh and V.S.Vihan, 2006. Polymerase chain reaction (PCR) for detection of *Br.melitensis* in goat milk. Small Ruminant Res., 65: 79-84.
31. Abd-El-Razik, K.A., H.M. Desouky and W.M. Ahmed 2007. Investi-gations on Brucellosis in Egyptian Baladi Does with emphasis on eval-uation of diagnostic techniques. Pak. J. Biol. Sci., 10 : 342-348.
32. Adams, L.G., 2002. The pathology of Brucellosis reflects the outcome of the battle between the host Genome and the Brucella genome. Vet. Microbiol., 90: 553-561.
33. Naroeni, A.N., J.S. Ouahrani-Bettache, J.P. Liautard and F. Pore, 2001. *Br. suis* -imparired specific recognition of phagosomes by lysosomes dues to phagosomal membrane modification. Infectious Immunol., 69: 486-493.
34. El-mahdy, M.M., Fatmam M. Darwish, F.B. Omnia and R.A.A. Eid, 2002. Pathological, bacteriological, parasitoloical studies on lymph nodes affection in cattle. Egyptian J. Comparitive Clin. Pathol., 15: 47-67.
35. Jones, T.C. and R.D. Hunt, 1998. Veterinary pathology. 4th Edn. Williams and Willikins A Waverly Company.
36. Palmer, M.V., N.F. Cheville and F.M. Tatum, 1996. Morphometric and histopathologic analysis of lymphoid depletion in murine spleens following infection with *Br. abortus* strains 2308 and RB51 or an htr A deletion mutant. Vet. Pathol., 33: 282-289.
37. Cheville, N.F., A.F. Olsen, M.G. Jensen, Stevens, A.M. Florance and H.S. Houng, 1996. Bacterial persistence and immunity in goats vaccinated with a pur E deletion mutant or the parental 16M strain of *Brucella melitensis*. Infectious Immunol., 64: 2431-2439.
38. Meador, V.P. and B.L. Deyoe and N.F. cheville, 1989. Pathogenesis of Brucella abortus infection of the mammary gland and supramammary lymph node of the goats. Vet. Pathol., 26: 357-368.

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