**Khalafallah, S. S., Hoda, K. Z. and Seada, A. S. H (2020): Some epidemiological studies on brucellosis in dairy farms in Gharbia Governorate. Egypt. Benha Veterinary Medical Journal.**

**Abstract:**

This study was conducted on dairy farms in Gharbia governorate, Egypt. From January to December 2018 .The study included 240 dairy farms and 3775 serum sample were collected for serological tests.25 farms (10.42) were positive for brucellosis,132 serum sample were positive by serological test divided according to production stage of animals as 121/3000(4.033%), 8/600 (1.33%) and 2/175 (1.142%) in cows ,heifers and bulls respectively. The result of culturing and confirmation by PCR indicated that *Brucella melitensis* biovar 3 is the dominant strain in dairy farms in Gharbia governorate. The spatial distribution of positive cases indicated that districts of Samnood, Kfr- Elzyat and Basion had the higher percent of positive cases as 6.35%, 4.67% and 3.57% respectively, however districts of El-Santa, Zefta and El-Mahla El-Kobra had the lowest percent as 1.49%, 2.3% and 2.89% respectively. The obtained result proved that, brucellosis is endemic in Gharbia governorate and good control program should be conducted to eradicate the disease.

**Key words**: *Brucella*, prevalence, dairy farm, spatial, temporal.

## 1.INTRODUCTION

Brucellosis is an important zoonotic disease that infects both livestock and human in many developing countries (Boschiroli *et al*., 2001).

Brucellosis is a reproductive syndrome, with clear signs as abortion, retained foetal membranes and low fertility. Brucellosis in cow is caused principally by *Brucella abortus*, which comprises nine serotypes and a number of variant strains (Dobrean *et al*., 2002).

The disease of brucellosis is important because of its widespread distribution, multiplicity of hosts and its public health hazard (Refai, 2002).

*Brucella* species are facultative bacteria present intracellular in many organs in the body and induce the disease of brucellosis. It causes abortion in dairy cows and fever with arthritis and endocarditis in infected man. There are many vaccines for animals but till now not approved for human use (Wang and Wu, 2014).

Because brucellosis is related to breeding process in animals and the microorganism excreted in body fluids as vaginal and uterine secretions beside milk, so dealing with these substances should be with caution and under good hygienic practices (Shareef, 2006).

In Egypt, control of brucellosis consisted of two procedures; preventing the exposure of susceptible animals to infection through the application of hygienic measures and increasing the immunity of animal population through vaccination and slaughter of infected animals (Ragan *et al*., 2013). This study was done to investigate the epidemiological panel of brucellosis in dairy cattle farms in Gharbia governorate, Egypt.

## 2. MATERIAL AND METHODS

### *2.1. Study area:*


**Figure (1) map of study area (Gharbia governorate, Egypt).**

The site of Gharbia governorate is in the center of Nile Delta (SIS egy, 2018). According to the annual report of Ministry of Agriculture the total number of cattle was 224007animals.

*2.2. Samples:*

*2.2.1. Samples for serological investigation:*

3775 serum samples were collected from 240 dairy cattle farms in Gharbia governorate, Egypt. Serum samples were kept at -20 C° for serological tests (Alton *et al*., 1988).

*2.2.2. Samples for bacteriological examination:*

Few grams of tissue samples were collected from 52 slaughtered serological positive animals; (supra-mammary lymph nodes, spleen and liver) under complete aseptic conditions and packed in sterile plastic bags and kept in ice box during transportation to the laboratory for bacteriological examination.

*2.2.3. Samples for polymerase chain reaction:*

Tissues including; (lymph nodes, Liver and spleen) and whole blood samples were brought from slaughtered serological positive cows, into sterile bags and sterile heparinized vacutainer tube and were stored at-80 C° until using.

*2.3. Serological tests:*

*2.3.1. Serological examination:*

All serum samples were examined for *Brucella* antibodies by Buffer acidified plate test (BAPA), Rose Bengal plate test (RBPT), Tube agglutination test (TAT), Rivanol test (Riv.T) and Complement fixation test (CFT) as described by Alton *et al*., (1988).

All antigens were obtained from the Veterinary Sera and Vaccine Research Institute Abassia, Cairo, Egypt.

*2.4. Isolation of Brucella:*

Specimens were cultured on 8% blood agar media (Oxoid, CM 271) and *Brucella* specific media (Oxoid, CM 169) supplemented with *Brucella* Selective Supplements (Oxoid, SR209E).

Cultures were incubated at 37°C for 7 days aerobically and micro-aerobically under a tension of 10% $CO_2$ following the method of Ribierio and Herr, (1990).

*2.5. Polymerase Chain Reaction (PCR):*

Extraction and analysis of PCR samples were performed as mentioned with Bricker and Halling,(1995).

*A. DNA extraction.*

DNA extraction from blood samples; DNA was extracted from blood using Blood DNA preparation Kit (Jena Bioscience Cat. No. PP-205S) Primers.

*B. DNA Amplification.*

DNA amplification was done by different PCR sets of primers.

**Table (1): Sequences of oligonucleotide primers used for PCR.**

| PCR Identification | Primer and probe | Sequence (5' to 3') |
|---|---|---|
| *Brucella spp* | Forward primer 5´-3´ | GCT-CGG-TTG-CCA-ATA-TCA-ATG-C |
| | Reverse primer 5´-3´ | GGG-TAA-AGC-GTC-GCC-AGA-AG |
| | Probe 5´-3´ | 6FAM-AAA-TCT-TCC-ACC-TTG-CCC-TTG-CCA-TCA-BHQ1 |
| *B.abortus* | Forward primer 5´-3´ | GCG-GCT-TTT-CTA-TCA-CGG-TAT-TC |
| | Reverse primer 5´-3´ | CAT-GCG-CTA-TGA-TCT-GGT-TAC-G |
| | Probe 5´-3´ | HEX-CGC-TCA-TGC-TCG-CCA-GAC-TTC-AAT-G-BHQ1 |
| *B.melitensis* | Forward primer 5´-3´ | AAC-AAG-CGG-CAC-CCC-TAA-AA |
| | Reverse primer 5´-3´ | CAT-GCG-CTA-TGA-TCT-GGT-TAC-G |
| | Probe 5´-3´ | Cy5-CAG-GAG-TGT-TTC-GGC-TCA-GAA-TAA-TCC-ACA-HQ2 |

*C. Analysis of the PCR Products:*

Electrophoresis was used for separation of the products of PCR on 1 % agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at 25C° by using of gradients of 5V/cm for gel analysis, 15 µl of the products was loaded in each gel slot. A generuler 100 bp DNA Ladder (Fermentas, Thermo, Germany) was used for determination of the fragment sizes.

# 3. RESULTS

**Table (2) Results of different types of serological examination.**

| Examined animals | No .of examined animals | BAPAT | | RBPT | | TAT | | Riv. T | | CFT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive | | Positive | | Positive | | Positive | | Positive | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| **Cows** | 3000 | 128 | 4.267 | 124 | 4.133 | 122 | 4.067 | 121 | 4.033 | 121 | 4.033% |
| **Heifers** | 600 | 10 | 1.67 | 8 | 1.33 | 8 | 1.33 | 8 | 1.33 | 8 | 1.33% |
| **Males** | 175 | 5 | 2.86 | 4 | 2.28 | 4 | 2.28 | 2 | 1.142 | 2 | 1.142% |
| Total | 3775 | 143 | 3.78% | 136 | 3.6% | 134 | 3.55% | 131 | 3.47% | 131 | 3.47% |

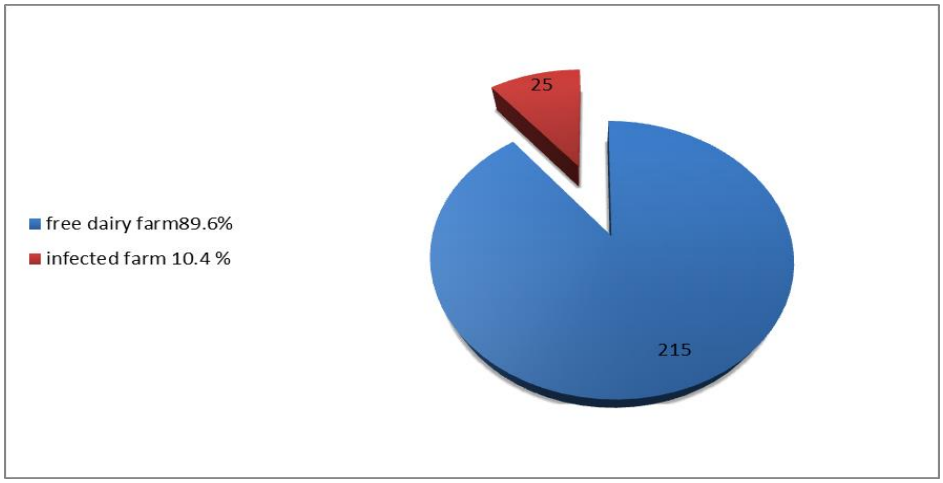


**Figure (2)** prevalence of brucellosis among dairy cattle farms in Gharbia governorate year 2018.

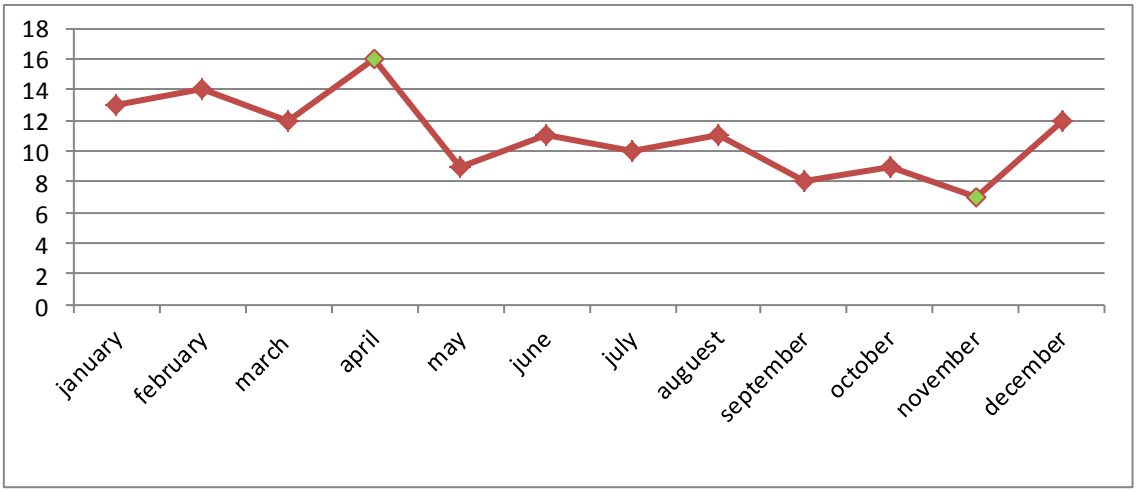


**Figure (3)** Temporal distribution of positive cases of dairy farms in Gharbia governorate.

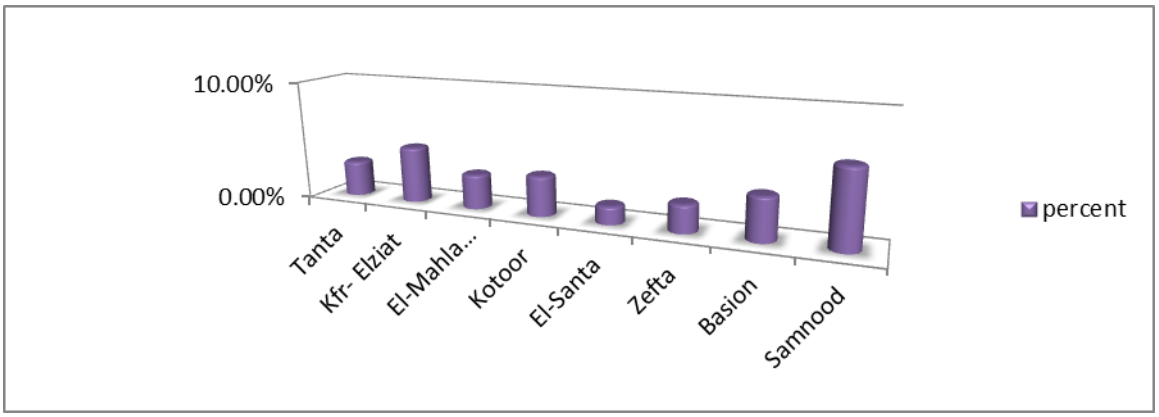


**Figure (4)** Spatial distribution of positive cases of dairy farms in Gharbia governorate in year 2018.

**Table (3)** Results of isolation and identification of *Brucella* organism from lymph nodes and organs of examined animals

| examined animals | Number | Supra-mammary L. n | | Spleen | | Liver | | Type of isolates |
|---|---|---|---|---|---|---|---|---|
| | | No. of positive | % | No. of positive | % | No. of positive | % | |
| **Cows** | 48 | 29 | 60.41 | 25 | 52.08 | 19 | 39.58 | ***Br. melitensis* biovar 3** |
| **Heifers** | 4 | 3 | 75.00 | 2 | 50.00 | 2 | 50.00 | |

Serological examination of 240 dairy farms (3775 serum samples) by using different serological tests, sero-positivity was obtained in 3.7% (140 ⁄ 3775), 3.65% (138 ⁄ 3775), 3.65% (138 ⁄ 3775), 3.47% (131/3775) and 3.49% (132 ⁄ 3775) using BAPA, RBPT, TAT, RivT and CFT of samples respectively. 3775 animals were examined for brucellosis from January to December year 2018 by serological methods and the results were 121 (4.033%), 8(1.3%) and 3 (1.7%) in cows, heifers and males respectively as showed in Table (2). Twenty five farms (10.4%) were infected with brucellosis and 215 farms (89.6%) were free as showed in Figure (2).

The result analysis indicated that, April, February and January had higher rate of positive cases as 16, 14, and 13 positive cases respectively. However September and November had lower rate as 8 an7 positive cases respectively as showed in Figure (3) .Also results showed that, districts of Samnood, Kfr-

Elzyat and Basion had the higher percent of positive cases as 6.35%, 4.67% and 3.57% respectively, however districts of El-santa , Zefta and El-Mahla El-Kobra had the lowest percent as 1.49% , 2.3% and 2.89% respectively as in Figure (4).

Confirmatory diagnosis by the isolation of etiological agent results showed in Table (3) revealed that the rate of isolation from examined supra-mammary lymph nodes, spleen and liver were 61.54%, 40.38% and 36.54%, all typed as *Brucella melitensis* biovar 3.

PCR used for more confirmation of bacteriological isolates and all isolates gave positive results at band 731 bp (*Brucella melitensis* bio var 3) as showed in Figure (5)

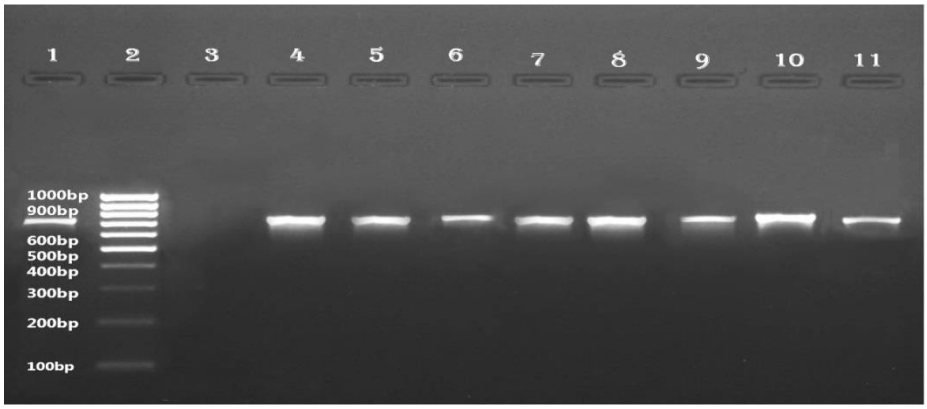


**Figure (5)** Result of PCR and electrophoreses Ethidium bromide stained 2 % agarose gel of PCR products showed +ve control (Lane 1), base indicator (Lane 2), –ve control (Lane 3) and *Brucella melitenses* +ve samples (lanes 4-11) of 731 bp PCR products. M represents a 100-bp ladder as a size standard.

## 4. DISCUSSION

Bovine brucellosis is a great problem in dairy cattle farms as it causes abortion in dairy animals in many countries in the world. The resistance of animals to *Brucella* infection is correlated with sex, age and reproductive status of the animals (Ducrotoy *et al*., 2018).

Multiple serological examinations should be used for the diagnosis of brucellosis because infected animal may not produce all antibody types in detectable levels (Alton *et al*., 1988).

In this study examination of serum samples with BAPAT, RBT, Riv. T .TAT and CFT. Seropositivity was obtained in 3.7% (140 ⁄ 3775), 3.65%

(138 ⁄ 3775), 3.65% (138 ⁄ 3775), 3.47% (131/3775 and 3.49% (132 ⁄ 3775) of samples respectively (Table 2).

The variation between the results of these tests was also reported by many authors (Moyer *et al*., 1987; Baum *et al*., 1995 and Shalaby *et al*., 2003). It can't depend on one type of serological test to diagnose of tested samples because many types of bacteria have antigen similar to *Brucella* as *Yersinia* and *E-Coli,* and that would give false positive results (Garin-Bastuji *et al*., 2006).

These highlight results indicated the necessary of using more than one type of diagnostic technique for the detection of positive animals for brucellosis, especially with epidemiological purposes. CFT is believed as gold standard test for detection of brucellosis because it can detect only antibodies type G that are specific for *Brucella* infection so it avoid the misdiagnosis due to the similar gram negative bacteria and so no false results detected (OIE 2009).

By serological surveillance in 240 dairy farms in the mentioned area, 25 farms were infected with brucellosis (10.42 %) as showed in Figure(2). From previous result we estimated that brucellosis is wide spread between dairy farms in Gharbia districts and endemic in this area. By testing of 3775 blood samples of dairy cows 132 animals were seropositive to brucellosis (3.49%) and the result were121 (4.033%), 8(1.3%) and3 (1.7%) in dairy cows, heifers and males respectively as showed in Table (2). According to this result, adult dairy cows have higher rate of infection because they have active reproductive system, that agree with a cross-sectional study that was conducted in same Governorate, in which the proportions of seropositive sera was 16% among livestock (El Sherbini *et al*., 2007). The rate of seropositive cases in buffaloes, goats, cattle and sheep for brucellosis is in Nile Delta was 5.7%, 5.9%, 7.3% and 11.4%, respectively (Sayour and Azzam, 2014). A previous study in the same Governorate found that, keeping different species of animals in same place as sheep with cattle was a highly risk factor for endemicity of brucellosis ($P$=0.01) and among livestock, cattle had the greatest seropositive rate of brucellosis (Hegazy *et al.,* 2011).

However another researches indicated higher prevalence of brucellosis inside the herds of cattle was 17.22% and the seropositive ratio in blood

samples was 2.16% (Kaoud *et al.,* 2010).The national records of animals services authority indicated that the prevalence of brucellosis in dairy cattle in Nile Delta was less than 0.5%, and more investigation was recommended to more accuracy in the results (Wareth *et al.*, 2014).

Results of culturing of tissue samples from lymph nodes, spleen and liver were 61.54%, 40.38% and 36.54% respectively. These findings come in accordance with previous results (Esmail *et al*., 2008). On the other hand, a higher rate of isolation of *Brucella* organism from supramammary L.Ns was 70% as reported by Laing *et al*., (1988).

*Brucella* organisms firstly localizing in regional lymph node then it proliferate within reticulo-endothelial cells then spread in body organs and localized inside it and can be isolated from liver, spleen and reproductive organs (Foster *et al*., 2017). All of the isolated strains were identified and biotyped by standard techniques as *Brucella melitensis* biovar 3. The obtained results were agreed with (Nielsen& Duncan., 1988). Who mentioned that direct culture methods usually are positive in 1-30% of cases. Also agree with previous results (Zahran .2004; Sleem, 2005 and Khoudair *et al.* 2009). Who isolated *Brucella melitensis* biotype 3 from different animal's species in Egypt and recorded that *Brucella melitensis* biotype 3 was the sole type in Egypt. There many factors affect the isolation process of *Brucella* microbe as purity of samples, number of living bacteria inside specimens, suitable laboratory conditions and good qualified personnel (Nielsen *et al*., 2004).

The reason of the isolation of *Brucella melitensis* biovar 3 from cattle may be attributed to the nearly constant close contact with infected sheep and goats. These findings have a great epidemiological importance as *Brucella melitensis* is more dangerous for human than other *Brucella* species (Alton *et al*., 1988).

The low recovery rates of *Brucella* from different samples obtained from sero-positive animal species by using traditional methods of isolation because *Brucella* is intracellular presenting bacteria and with temporary shedding in animal secretion so it need the using of more advanced tools like PCR. However that isolation of *Brucella* still more accurate confirmatory method for diagnosis of the disease (Neta *et al.,* 2010).

Blood samples were analyzed by PCR and electrophoresis techniques to more confirmation and to more detection of the species and biovar. All

*Brucella* strains gave 731 bp *Brucella melitensis* species bands biovar 3 as showed in Figure (5). In this research we depended on fact that molecular detection of *Brucella* infection can be done directly on clinical samples without previous isolation of the organism. In addition, these techniques can be used to complement results obtained from phenotypic tests. Polymerase Chain Reaction (PCR) and its variants based on amplification of specific genomic sequences of the genus species or even biotypes of *Brucella* spp. are the most broadly used molecular technique for brucellosis diagnosis (Leal-Klevezas *et al*., 1995; Xavier *et al*., 2010). *Brucella melitensis biotype 3* was the sole type in Egypt. Isolation of the living microbe is very critical process and need more precaution and biosecurity Brucella microorganism need specific condition for growth as supplements and $co_2$ tension (Nielsen *et al*., 2004).

The results agree with results obtained by Wareth *et al*.,(2015) Who reported that PCR must be considered an alternative to the traditional culturing methods for *Brucella* diagnosis as screening and confirmatory diagnostic tool for saving cost and time.The obtained results were similar to that recorded by Ahmed *et al.*, (2012) who reported that PCR is the highest sensitive method which makes the detection of nucleic acid of *Brucella* achievable. The results can be obtained rapidly so that they can be used not only to support bacteriological investigation but also to make them reliable.

## 5.Conclusions:

Brucellosis was endemic in dairy farms in Gharbia governorate Egypt. The district of Samnod had higher rate of positive cases however El-Santa district had lesser rate. The major rate of positive cases was in cold season and decreased at hot months. *Brucella melitinses* biovar 3 was the isolated strain that indicated the mixing housing and the close contact between cattle and sheep was the most risk factor for the disease.

## 6. References:

Ahmed, Y. F., Sokkar, S. M., Desouky, H. M., & Madbouly, A., 2012**.** Pathological studies on buffalo-cows naturally infected with Brucella melitensis. *Global Vet*, *9*(6), 663-668.

Alton,G.G.,Jones,R.D.Angus,&.Verger,M,J.,1988.Techniques for the brucellosis laboratory. Institut Nationaldela Recherche Agronomique, Paris, France, 19.(2)213-220.

Baum, M., Zamir, O., Bergaman-ios, R., Katz, E., Beider, Z., Cohen, A. and Banai, M., 1995. Comparative evaluation of micro-agglutination test and serum agglutination test as supplementary diagnostic methods for brucellosis. J. Clin. Micobiol., 33 (8): 2166-2170.

Boschiroli M.L., Foulongne V. & O'Callaghan D., 2001. Brucellosis: a worldwide zoonosis. Curr. Op. Microbiol.,4 (1), 58-64).

Bricker, B. J., & Halling, S. M., 1995. Enhancement of the Brucella AMOS PCR assay for differentiation of Brucella abortus vaccine strains S19 and RB51. *Journal of Clinical Microbiology*, *33*(6), 1640-1642.

Dobrean V., Opris A. & Daraban S., 2002. An epidemiological and surveillance overview of brucellosis in Romania. Vet. Microbiol., 90 (1-4), 157-163.

Ducrotoy, M. J., Muñoz, P. M., Conde-Álvarez, R., Blasco, J. M., & Moriyón, I., 2018. A systematic review of current immunological tests for the diagnosis of cattle brucellosis. *Preventive veterinary medicine*, *151*, 57-72.

El Sherbini, A., Kabbash, I., Schelling, E., El Shennawy, S., Shalapy, N., Elnaby, G. H & Eisa, A., 2007. Seroprevalences and local variation of human and livestock brucellosis in two villages in Gharbia Governorate, Egypt. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *101*(9), 923-928.

Esmaeil, Z., Abdollah, E.,& Mehran, Y., 2008. Isolation and identification of Brucella organisms in Iran. Iranian. J. of Clinic Infectious Diseases. 3(4):185-188.

SIS egy, 2018. web site. https://www.sis.gov.eg/section/10/20_lang = Gharbia / governorate.com.

Foster, J.T, Walker, M.F., Rannals, D.B., Hussain, H.M., Drees, P.K., Tiller., V.R., Hoffmaster, R.A., Al-Rawahi, A., Keim, P.,& Saqib, M., 2017. African lineage Brucella melitensis isolates from Omani livestock. Front. Microbiol. 8, 2702.

Garin-Bastuji, B., Blasco, J.M., Mar´ın, C. & Albert, D., 2006. The diagnosis of brucellosis in sheep and goats, old and new tools. Small Ruminant Research, 62: 63–70.

Hegazy, Y. M., Molina-Flores, B., Shafik, H., Ridler, A. L., & Guitian, F. J., 2011. Ruminant brucellosis in upper Egypt . *Preventive veterinary medicine*, *101*(3-4), 173-181.

Kaoud, H. A., Zaki, M. M., El-Dahshan, A. R., & Nasr, S. A., 2010. Epidemiology of brucellosis among farm animals. *Nature and Science*, *8*(5), 190-197.

Khoudair, R. M., Ibrahim, E. M., Saker, G. G., & Hafez, M. A., 2009. Clinicodiagnostic and pathological studies on cattle and buffaloes suffering from brucellosis and tuberculosis in Kafr El Sheikh Governorate. *Egyptain Journal of Comparative Pathology and Clinical Pathology*, *22*(1).

Laing, J. A.; Morgan, W. J. & Wanger, W. C., 1988. Brucellosis in fertility and infertility in veterinary practice. Corbel, M. J. Edited by English Book Language book society, Bailliere, Tindall, Pp: 189-220.

Leal-Klevezas, D. S., Martínez-Vázquez, I. O., Lopez-Merino, A., & Martínez-Soriano, J. P., 1995. Single-step PCR for detection of Brucella spp. from blood and milk of infected animals. *Journal of clinical microbiology*, *33*(12), 3087-3090.

Moyer, N.P., Evis, G.M., Pigott, N.E., Hudson, J.D., Farshy, C.E., Feeley, J.C. & Hausler, J.W., 1987. Comparison of serologic screening tests for brucellosis. J. Clin. Microbiol., 25 (10): 1969-1972.

Nakouné E., Debaere O., Koumanda-Kotogne F., Selekon B.,Samory F. & Talarmin A., 2004. Serological surveillance ofbrucellosis and Q fever in cattle in the Central AfricanRepublic. Acta trop., 92 (2), 147-151.

Neta, A. V. C., Mol, J. P., Xavier, M. N., Paixão, T. A., Lage, A. P., & Santos, R. L., 2010. Pathogenesis of bovine brucellosis. *The Veterinary Journal*, *184*(2), 146-155.

Nielsen, K., & Duncan, J. R., 1988. Antibody isotype response in adult cattle vaccinated with Brucella abortus S19. *Veterinary immunology and immunopathology*, *19*(3-4), 205-214.

Nielsen, K., Smith, P., Widdison, J., Gall, D., Kelly, L., Kelly, W., & Nicoletti, P., 2004. Serological relationship between cattle exposed to Brucella abortus, Yersinia enterocolitica O: 9 and Escherichia coli O157: H7. *Veterinary microbiology*, *100*(1-2), 25-30.

OIE, 2009. "Terrestrial manual, Bovine brucellosis," in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, vol. 2, chapter 2.4.3, pp. 624–659, OIE, Paris, France.

Ragan, V., Vroegindewey, G., & Babcock, S., 2013. International standards for brucellosis prevention and management. *Rev Sci Tech*, *32*(1), 189-98.

Refai M, 2002. Incidence and control of brucellosis in the Near East region. Vet. Microbiol., 90 (1-4), 81-110.

Ribeiro, L. M. M., & Herr, S., 1990. The use of filter paper discs impregnated with thionin acetate, basic fuchsin and thionin blue in the identification of *Brucella* species.

Sayour, A. E., & Azzam, R. A., 2014. Epidemiological characterization and pathogenicity of prevalent Brucella strains in Egypt. *Annals of Veterinary and Animal Science, eISSN*, 2313-5514.

Shalaby, M.N.H., Ghobashy,H.M., El-Bauomey, E.M., & Saleh, Wafaa, M.A., 2003. Prevalence of brucellosis among farm animal species in some Governorates in Egypt. Proceeding of the seventh scientific congress of the Egyptian Society for cattle diseases 28(2), 288-292.

Shareef, J. M., 2006. Isolation of Brucella abortus from aborted lambs in the governorate of Sulaimania/Said-Sadic District. *Iraqi Journal of Veterinary Sciences*, *20*(1), 19-23.

Sleem, Y. A., 2005. Bacteriological studies on *Brucella* microorganisms in sheep and goats in Sharkia Governorate.

Wang, Z., Wang, S., & Wu, Q., 2014. Cold shock protein A plays an important role in the stress adaptation and virulence of *Brucella* melitensis. *FEMS microbiology letters*, *354*(1), 27-36.

Wareth, G., Hikal, A., Refai, M., Melzer, F., Roesler, U., & Neubauer, H., 2014. Animal brucellosis in Egypt. *The Journal of Infection in Developing Countries*, *8*(11), 1365-1373.

Wareth, G., Melzer, F., Tomaso, H., Roesler, U., & Neubauer, H., 2015. Detection of Brucella abortus DNA in aborted goats and sheep in Egypt by real-time PCR. *BMC research notes*, *8*(1), 212.

Xavier, M. N., Silva, T. M., Costa, E. A., Paixao, T. A., Moustacas, V. S., Júnior, C. A. C& Tsolis, R. M., 2010. Development and evaluation of a species-specific PCR assay for the detection of Brucella ovis infection in rams. *Veterinary microbiology*, *145*(1-2), 158-164.

Zahran, A, E., 2004. *Bacteriological and serological studies on Brucella microorganisms in farm animals in El-Minia governorate* (Doctoral dissertation, PhD thesis, Department of Bacteriology).