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Some epidemiological studies on brucellosis in dairy farms in Gharbia governorate, Egypt

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ABSTRACT

This study was conducted on dairy farms in Gharbia governorate, Egypt from January to December 2018. 240 dairy farms were included, 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 classified according to the productive stage of animals as 121/3000 (4.033%), 8/600 (1.33%) and 2/175 (1.142%) in cows, heifers and bulls, respectively. The culturing and PCR confirmation results indicated that *Brucella melitensis* biovar 3 was the dominant strain in the tested dairy farms. The spatial distribution of positive cases indicated that districts of Samnood, Kfr-Elzyat and Basion had the highest rate of positive cases (6.35%, 4.67% and 3.57%, respectively), yet El-Santa, Zefta and El-Mahla El-Kobra districts showed the lowest rates (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.

1. INTRODUCTION

Brucellosis is an important zoonotic disease that infects both livestock and human in many developing countries (Boschiroli et al., 2001). This disease is of important because of its widespread distribution, multiplicity of hosts and its public health hazard (Refai, 2002).

Brucellosis is a reproductive syndrome with clear signs of abortion, retained fetal membranes and low fertility. In cows, it is caused principally by Brucella abortus, which comprises nine serotypes and a number of variant strains (Dobrean et al., 2002).

Brucella species are facultative bacteria present intracellular in many body organs 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 is excreted in body fluids e.g. vaginal and uterine secretions and milk, so dealing with these substances should be with caution and under good hygienic practices (Shareef, 2006).

In Egypt, control of brucellosis depends on 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

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

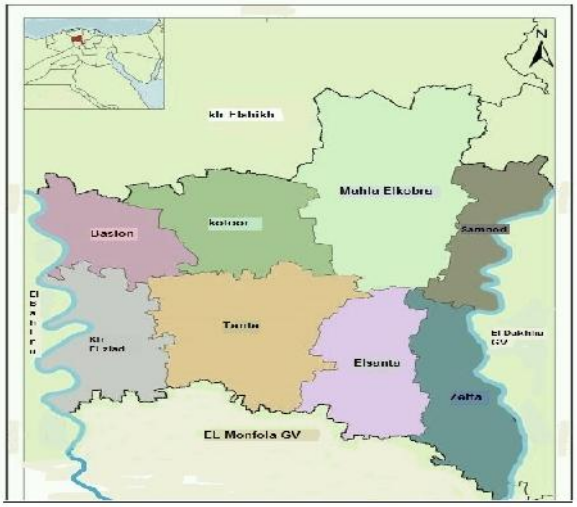

Figure 1 Map of study area (Gharbia governorate, Egypt).

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

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kept at -20 °C for serological tests (Alton *et al*., 1988).

2.2.2. Samples for bacteriological examination:

Tissue samples were collected from 52 slaughtered serological positive animals from supra-mammary lymph nodes, spleen and liver under complete aseptic conditions and were 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 samples including lymph nodes, Liver and spleen, and whole blood 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).

i. DNA extraction.

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

ii. DNA Amplification.

DNA amplification was done by different PCR sets of primers.

iii. Analysis of the PCR Products:

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

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

3. RESULTS

Serological examination of 240 dairy farms (3775 serum samples) by using different serological tests showed that the 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. Cows, heifers and bulls examined for brucellosis and showed positivity were 121 (4.033%), 8(1.3%) and 3 (1.7%), respectively (Table 2). Twenty-five farms (10.4%) were infected with brucellosis and 215 farms (89.6%) were free as showed in fig. 2.

The data analysis indicated that April, February and January had the highest positive rate as 16, 14, and 13 positive cases, respectively. However, September and November had the lowest rate as 8 an 7 positive cases, respectively (Figure 3).

The results showed that Samnood, Kfr-Elzyat and Basion districts had the highest rate of positive cases as 6.35%, 4.67% and 3.57%, respectively. Meanwhile, El-santa, Zefta and El-Mahla El-Kobra districts 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 as shown 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).

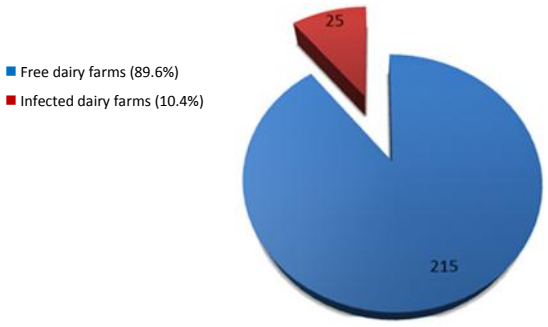

Figure 2 Prevalence of brucellosis among dairy cattle farms in Gharbia governorate year 2018.

Table 2 Results of different types of serological examination.

Examined animals	No. of examined animals	BAPAT Positive		RBPT Positive		TAT Positive		Riv. T Positive		CFT Positive	
		n	%	n	%	n	%	n	%	n	%
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%

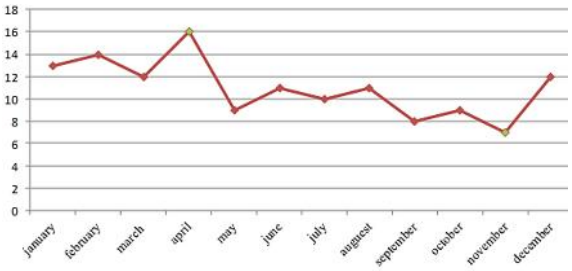

Figure 3 Temporal distribution of positive cases of dairy farms in Gharbia governorate.

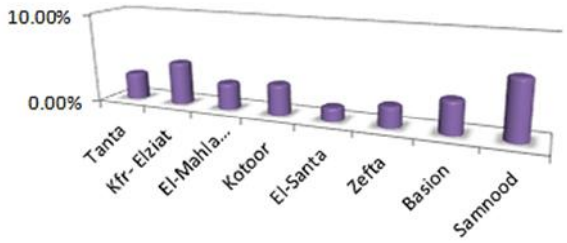

Figure 4 Spatial distribution of positive cases of dairy farms in Gharbia governorate in year 2018.

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

examined animals	Number	Supra-mammary L. n		Spleen		Liver		Type of isolates
		n	%	n	%	n	%	
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	

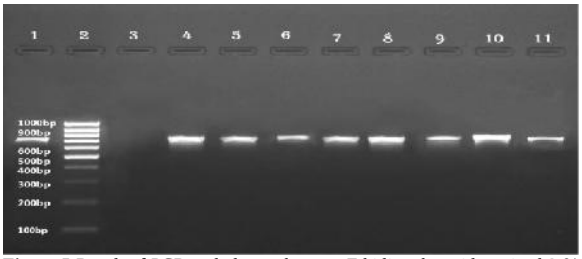

Figure 5 Result of PCR and electrophoreses Ethidium bromide stained 2 % agarose gel of PCR products showed +ve control (Lane 1), base indicator (Lane 2), –ve control (Lane 3) and *Brucella melitenses* +ve samples (lanes 4-11) of 731 bp PCR products. M represents a 100-bp ladder as a size standard.

4. DISUCSSION

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; 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 widespread 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%) and 3 (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 supra-mammary L. Ns was 70% as reported by Laing *et al*. (1988).

Brucella organisms firstly localizing in regional lymph node then it proliferates 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*., 2018). All of the isolated strains were identified and bio-typed by standard techniques as *Brucella melitensis* biovar 3. The obtained results were agreed with (Nielsen and Duncan., 1988), Who mentioned that direct culture methods usually are positive in 1-30% of cases. Also agreed with previous results (Zahran, 2004; Sleem, 2005; 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.

5. CONCULOSION

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 melitensis* 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.

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