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Identification of *Brucella* spp. and Assessing Impact of Brucellosis Control Programme on Ruminants and Human in Gharbia Governorate, Egypt

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ABSTRACT

The aim of the present study was to assess the temporal impact of brucellosis control programme on ruminants and human and to identify *Brucella* spp. in Gharbia governorate, Egypt. Data for brucellosis in ruminants were collected from the active surveillance programme for brucellosis. Blood and tissue (lymph nodes and spleen) samples from positive animals were also collected. Data for human cases were obtained from the Ministry of Health, Gharbia governorate, Egypt. Statistical analyses were conducted to allow the comparison between different years and ruminant species. To compare between seropositive proportions for different years for each species, a univariate binary logistic regression model was used. There was no consistency in sampling and testing of animals and less than 3% were tested in any given year and the highest proportion of animals tested were cattle. There were variations in seropositive proportions in different species of tested animals and between districts. The number of reported cases of brucellosis in humans was increasing and there was a positive association with that in ruminants. About 36% and 50% of lymph nodes and spleen samples were culture positive, respectively. All isolated strains were identified as *B. melitensis biovar 3*. Brucellosis is an endemic disease in the study area and the current control programme (test and slaughter) doesn't seem to be effective. Further studies are required for assessing the social and economic impacts of brucellosis. This study indicated that the impact of the current control programme of brucellosis in an endemic area of Egypt. The outcomes of this study would help policy makers to rethink about the control of brucellosis and look for alternative strategies.

Keywords: Brucellosis, Ruminants, Human, Nile Delta, Egypt

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INTRODUCTION

Brucellosis is one of the most common zoonotic diseases worldwide. The disease is endemic and a major cause of economic losses particularly in developing countries (McDermott et al., 2013; Refai, 2002; Pappas and Memish, 2007). This may be due to the lack of resources, lack of compliance to control programmes and the attitudes of livestock owners (Hegazy et al., 2009; Holt et al., 2011 and Eltholth et al., 2015). Since brucellosis was reported in Egypt in 1939, the disease has been endemic in the country with a high prevalence. The national control programme for brucellosis in ruminants since 1981involves testing all females older than six months and slaughtering the serologically positive ones, with voluntary vaccination of calves using *Brucella abortus S19* vaccine also lambs and kids by *Rev1* vaccine (Hassanain and Ahmed, 2012;Refai, 2002). Evaluation of the impact of the disease control in El-Beheira governorate, Egypt, using data from the active surveillance programme between 1990 and 2012 indicated that brucellosis was endemic and there was no significant reduction of the seropositive animals (Eltholth et al., 2015). Further analysis of the impact of the control programme of brucellosis on the seroprevalence in ruminants all over the country between 1999 and 2011 indicated that the disease is endemic without significant reduction the seropositive proportion of ruminants (Eltholth et al., 2016).

Although data for the prevalence of brucellosis in Egypt are scarce, the following studies suggested the disease is endemic in all ruminant species, with a high prevalence. In a study where samples were collected from 126 herds from all over the country, 26.66%, 18.88% and 17.22% of sheep flocks, goat flocks and cattle herds were seropositive for *Brucella spp*. respectively (Kaoud et al., 2010). In some regional studies such as in Monufia governorate, 5.36%, 3.33%

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and 3.17% of cattle, buffaloes and goats were seropositive for *Brucella spp*. by Rivanol test and 7.14%, 4.26%, 2.47% and 6.35% of cattle, buffaloes, sheep and goats were seropositive by the buffered acidified plate antigen, respectively (Samaha et al., 2008). In the same governorate, the individual and household seroprevalence of *Brucella spp.* in cattle and buffaloes was 11.0% and 15.5%, respectively (Holt et al., 2011). In Kafrelsheikh governorate, the seroprevalence in cattle, buffaloes, sheep and goats was at 12.2%, 12%, 12.2% and 11.3%, respectively (Hegazy et al., 2011). Recently, the seroprevalence of *Brucella* was reported to be18.09% in blood samples from sheep and goat flocks from five governorates in the Nile Delta (Mahboub et al., 2013).Samples from private dairy cattle farms in Dakahlia, Damietta and Port-Said governorates, indicated that the proportion of seropositive animals was 52.2% and 4.2% in cattle with reproductive failures and apparently healthy cattle, respectively (Gwida, 2015).

However, results from the previous study were not reliable to estimate the seroprevalence of brucellosis in the study area because of the lack of study design and targeting cattle with a history of reproductive failure. The most recent study in Kafrelsheikh governorate indicated that, the true seroprevalence of brucellosis in sheep was 20 % (95 % CI 15.3–24.7) with the village prevalence, having at least one seropositive sheep, of 95.5 % (95 % CI 92.2–100) and the village flock seroprevalence ranged from 0 to 46.8 % (Hegazy et al., 2016).These studies indicated that, brucellosis is endemic in Egypt with a high prevalence. The same situation in humans, there were no accurate data for the actual prevalence and/or incidence of brucellosis. Therefore it is important to assess the impacts of the current brucellosis control programme on the disease prevalence. The aim of this study was to assess the temporal impacts of brucellosis control programme on animals and human brucellosis in one of the central governorates of the Nile Delta of Egypt, Gharbia governorate. It was also important to identify *Brucella* spp. circulating in livestock. Therefore, blood and tissue samples from brucellosis positive animals were analysed.

MATERIALS AND METHODS

Study area

This study was conducted in Gharbia governorate located in the middle of the Nile Delta, between Damietta and Rosetta branches. In the north, it is bordered by KafrElshiekh, in the south by Monufia, in the east by Dakahlia, and in the west by El-Beheira governorate, figure 1. According to the 2014 censuses, it is one of the highly populated areas in Egypt with human population of 4,648,408 and a density of 2400/Km^2, Egypt State Information Service (ESIS). This governorate is also characterised by agricultural activities and a high density of animal population. About 60% of the inhabitants live in rural community in close contact with their livestock. According to the Ministry of Agriculture and Land Reclamation (MALR, 2010), the total number of cattle, buffalo, sheep and goat was 233,007, 245,408, 200,086 and 116,604, respectively. The number of animals for the subsequent years was estimated by using a growth rate of 1.1%. The proportion of animals supposed to be tested (aged more than 6 months) according to the brucellosis control programme in Egypt was estimated as 0.7 of the total number of animals.

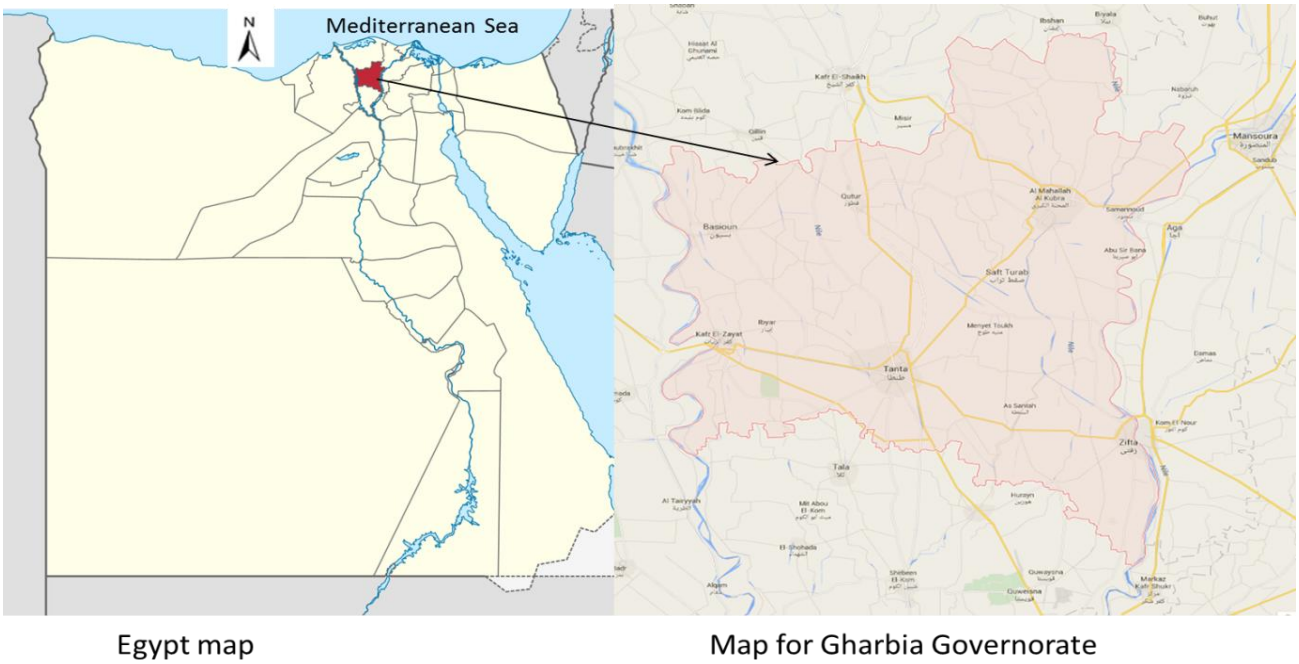

Figure 1. Study site for brucellosis in Gharbia governorate, Egypt

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Ethical approval

Handling of animals were according to the guidelines of Animal ethics committee, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt

Data for brucellosis in ruminants

The data for this study was collected from the active surveillance programme for brucellosis control conducted by the General Organisation of Veterinary Services (GOVS), Egypt. The available data were for years 2010 to 2015 for the annual number of animals tested and number positive for cattle, buffalo, sheep and goat. Detailed data per district were available only for the years 2010 to 2011.

Biological samples

Blood and tissue samples were collected from seropositive animals for brucellosis. These samples were used for Polymerase Chain Reaction (PCR) and bacteriological examination to identify *Brucella* spp. in Gharbia governorate. Blood samples (5ml) were collected from the jugular vein of the examined animals through a sterile dry needle into a sterile heparinized vacationer tube and stored at-80^{o}C until analyzed. Tissue samples (61 supramammary lymph nodes and 10 spleens) from different animal species were collected (Table 1). Tissue samples were collected and kept in sterile bags directly after slaughtering and stored at -20ºC until cultured.

Table 1. Bacteriological examination for presence of *Brucella* spp. in tissue samples of livestock in Gharbia governorate, Egypt.

Species	Lymph nodes	Spleen	Total
Cattle	23	4.0	27
Buffalo	15	2.0	17
Sheep	12	2.0	14
Goat	11	2.0	13
Total	61	10	71

Bacteriological examination and Polymerase Chain Reaction (PCR)

Isolation, identification and biotyping of *Brucella*spp. were carried out according to the recommendation of the FAO/WHO, expert committee on brucellosis(Alton et al., 1988). DNA was extracted from blood samples using blood DNA preparation kit(Jena Bioscience Cat. No. PP-205S) following the instruction of the manufacturer. DNA amplification was done by two different PCR sets of primers. Oligonucleotide primers specific for *B.abortus* were used to amplify the insertion sequences IS711(Bricker and Halling, 1994).Oligonucleotide primers P1 and P2 specific for *B. melitensis* were designed from *Brucella*omp 2 gene (Bardenstein et al., 2002). The sequences of the primers were listed in table 2. DNA amplification: the PCR 25 µL reaction volume containing 5 µLofTaq master ready–to use mixes for PCR (Jena Bioscience, Cat. No.1025), 10 PM of each oligonucleotides primer, 5 µL of DNA template and fill up to25 µL with DNAse-RNAse free water. For P1 and P2 primers, PCR was performed as follows: 35 cycles of PCR with 1 cycle consisting of 20s at 95ºC for DNA denaturation, 1 min at 50ºC for primer annealing and 1 min at 72ºC for polymerase mediated primer extension. The last cycle included incubation of the sample at 72ºC for 7 min. Samples were considered positive when there was a single band of DNA at 282 bp. For IS711 and *B. abortus* primers, after an initial denaturation at 93ºC for 5 min, the PCR profile was set as follows: template denaturation at 95ºC for 1.25 min, primer annealing at 55.5ºC for 2 min and primer extension at 72ºC for 2 min, for a total of 35 cycles, with a final extension at 72ºC. Samples were considered positive when a single band of DNA at 498 bp. All PCR were performed in a DNA thermocycler (Perken Elmer model 9600).

Table 2. Sequences of oligonucleotide primers used for polymerase chain reaction

Primers name	Sequences	Amplified product	References
P1	5`TGGAGGTCAGAAATGAAC3	282 bp	(Bardenstein et al., 2002)
P2	5` GAGTGCGAAACGAGCGC3`		
B. abortus	5'- GAC GAA CGG AAT TTT TCC AAT CCC	498 bp	
IS711	5'- TGCCGA TCA CTT AAG GGC CTT CAT		

Data for human brucellosis

Data for human cases were obtained from the EgyptianMinistry of Health, Gharbia directorate for the years2014, 2015 and the first quarter of 2013. The only available data were the number of confirmed human cases.

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Statistical analysis

Data were stored in a Microsoft Office Excel 2007. Frequency tables were used to calculate the proportions of tested and the proportions of seropositive animals for *Brucella* spp. per year for each ruminant species. Statistical analyses were conducted to allow comparison between different years and ruminant species using IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY: IBM Corp). To compare between seropositive proportions for different years for each species, a univariate binary logistic regression model, with seropositive as the response variable and year 2010 was used as the reference.

RESULTS

The results (Table 3) indicated that there was no consistency in sampling and testing of animals. The highest proportion of animals tested was cattle, from about 3% to about 6% of the number of cattle supposed to be tested. The proportion of buffalo and sheep tested ranged from about 1.5% to about 2%. The lowest proportion of ruminant tested was goat about 1%. The proportion of seropositive animals per districts in 2010 and 2011 are summarised in table 4 and 5, respectively. The results showed that there were variations in seropositive proportions in different species of ruminates and between districts. In 2010, the highest proportion of seropositive cattle (1.69%) was in El-Mahalla El-Kubra and the highest proportion of seropositive buffalo (2.43%) and sheep (5.5%) was in Tanta (Figure 2).

In 2011, the highest proportion of seropositive cattle (0.72%), sheep (1.08%) and goat (33.33%) was in Tanta, while the highest proportion of seropositive buffalo (1.2%) was in Kafr El-Zayat (Figure 3). Results for the seropositive proportion of ruminants along the study period are summarised in figure 4. It showed that in 2011 and 2014 the highest proportion of seropositive animals was observed among goat. Results of regression analysis are summarised in table6. In cattle, there was a significant decrease in the proportion of seropositive animals in 2011 and 2012 followed by an increase in 2013 to 2015. The proportion of seropositive buffalo was fluctuating, there was a significant decrease in 2011 than an increase in 2012 and 2013 followed by a decrease in 2014 and 2015. Among sheep, there was a general decrease in the seropositive proportion. For goat, there were no positive cases among those tested in 2010 and 2013. In 2014, the proportion of seropositive goat was higher than in any other species along the study period. According to the health records in Gharbia Directorate, Ministry of Health, there were 54,217 and 342 reported human cases of brucellosis in the last quarter of 2013, 2014 and 2015, respectively. The distribution of cases per district (Figure 5) indicated that, the highest number was recorded in El-Mahalla El-Kubra district particularly in 2015. These figures indicated that the number of reported cases of brucellosis in humans was increasing (Figure 6). Also the number of reported cases in humans was increasing with that in ruminants (Figure 7). The monthly distribution of reported human cases (Figure 8) indicated that, the highest number of cases was in June and March for 2014 and 2015, respectively. On the other hand the highest total number of reported cases per month in ruminants within the study period was in May followed by February (Figure 9). Tissue samples from seropositive animals, 61 lymph nodes and 10 spleens were cultured for bacteriological analysis. The results (Table 7) showed that 36.07% and 50% of lymph nodes and spleen samples were positive, respectively. About 40% of samples from cattle, buffalo and sheep were positive while 30% of samples from goat were positive. All isolated strains were identified biochemically as *B.melitensis* biovar 3. All examined blood samples of infected animals reacted positively with DNA products with a molecular size of 282 pb, indicative of *B.melitensis* DNA as shown in figure10.

Table 3. Livestock census and proportion of animals tested for brucellosis control programme in Gharbia governorate, Egypt during 2010-2015

Species / Year	Cattle			Buffalo			Sheep			Goat			Ruminants		
	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %
2010	163105	8236	5.05	171786	2795	1.63	140060	2300	1.64	81623	535	0.66	556574	13866	2.49
2011	171950	8867	5.16	181102	3615	2.00	147656	1561	1.06	86049	470	0.55	586758	14513	2.47
2012	181276	4637	2.56	190923	2822	1.48	155664	3373	2.17	90716	868	0.96	618578	11700	1.89
2013	191106	7866	4.12	201277	2943	1.46	164106	968	0.59	95636	250	0.26	652125	12027	1.84
2014	201470	11589	5.75	212193	4320	2.04	173005	1991	1.15	100822	304	0.30	687491	18204	2.65
2015	212397	12386	5.83	223701	3799	1.70	182388	1980	1.09	106289	1115	1.05	724775	19280	2.66

N=number of animals tested/positive, %=percentage of animals tested/positive

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Table 4. Proportion of seropositive ruminates for brucellosis in Gharbia Governorate, Egypt, per district in 2010

Species / District	Cattle			Buffalo			Sheep			Goat			Total		
	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %
Tanta	1689	17	1.01	206	5.0	2.43	73	4.0	5.5	120	0.0	0.0	2088	26	1.25
Zifta	361	0.0	0.00	630	1.0	0.16	1.0	0.0	0.0	2.0	0.0	0.0	994	1.0	0.10
El-Mahalla El-Kubra	826	14	1.69	362	3.0	0.83	1218	18	1.5	2.0	0.0	0.0	2408	35	1.45
Kotoor	1263	0.0	0.00	253	0.0	0.00	120	0.0	0.0	0.0	0.0	0.0	1636	0.0	0.00
Kafr El-Zayat	1446	3.0	0.21	124	1.0	0.81	0	0.0	0.0	0.0	0.0	0.0	1570	4.0	0.25
El-Santa	1007	10	0.99	633	2.0	0.32	644	1.0	0.2	217	0.0	0.0	2501	13	0.52
Bassyoun	1335	3.0	0.22	84	1.0	1.19	194	4.0	2.1	78	0.0	0.0	1691	8.0	0.47
Samannoud	309	0.0	0.00	503	0.0	0.00	50	0.0	0.0	116	0.0	0.0	978	0.0	0.00
Total, n (%)	8236	47	0.57	2795	13	0.47	2300	27	1.2	535	0.0	0.0	13866	87	0.63

N=number of animals tested/positive, %=percentage of animals tested/positive

Table 5. Proportion of seropositive ruminates for brucellosis in Gharbia Governorate, Egypt, per district in 2011

Species / District	Cattle			Buffalo			Sheep			Goat			Total		
	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %
Tanta	1253	9.0	0.72	216	1.0	0.46	185	2.0	1.08	12	4.0	33.33	1666	16	0.96
Zifta	572	2.0	0.35	949	0.0	0.00	89	0.0	0.00	11	0.0	0.00	1621	2.0	0.12
El-Mahalla El-Kubra	768	0.0	0.00	447	0.0	0.00	125	0.0	0.00	0.0	0.0	0.00	1340	0.0	0.00
Kotoor	1486	0.0	0.00	276	0.0	0.00	98	0.0	0.00	0.0	0.0	0.00	1860	0.0	0.00
Kafr El-Zayat	2363	1.0	0.04	83	1.0	1.20	143	0.0	0.00	0.0	0.0	0.00	2589	2.0	0.08
El-Santa	1078	4.0	0.37	856	2.0	0.23	523	0.0	0.00	249	2.0	0.80	2706	8.0	0.30
Bassyoun	1010	1.0	0.10	164	0.0	0.00	228	0.0	0.00	198	2.0	1.01	1600	3.0	0.19
Samannoud	337	0.0	0.00	624	0.0	0.00	170	0.0	0.00	0.0	0.0	0.00	1131	0.0	0.00
Total, n (%)	8867	17	1.58	3615	4.0	1.90	1561	2.0	0.13	470	8.0	1.70	14513	31	0.21

N=number of animals tested/positive, %=percentage of animals tested/positive

Table 6. Proportion of seropositive ruminates for brucellosis in Gharbia Governorate, Egypt from 2010 to 2015

Animal spp.	Year	Tested animals	Seropositive (%)	OR	95% CI	P value
Cattle	2010	8236	0.57	Ref	-	-
	2011	8867	0.19	0.34	0.335-0.192	0.000
	2012	4637	0.13	0.23	0.096-0.528	0.001
	2013	7866	0.42	0.73	0.470-1.147	0.174
	2014	11589	0.53	0.94	0.641-1.371	0.738
	2015	12386	0.68	1.19	0.832-1.702	0.342
Buffalo	2010	2795	0.47	Ref	-	-
	2011	3615	0.11	0.24	0.077-0.728	0.012
	2012	2822	0.18	0.38	0.135-1.067	0.066
	2013	2943	0.34	0.73	0.319-1.667	0.454
	2014	4320	0.16	0.35	0.138-0.872	0.024
	2015	3799	0.21	0.45	0.187-1.091	0.077
Sheep	2010	2300	1.17	Ref	-	-
	2011	1561	0.13	0.11	0.026-0.455	0.002
	2012	3373	0.71	0.60	0.347-1.048	0.073
	2013	968	0.31	0.26	0.079-0.865	0.028
	2014	1991	0.50	0.42	0.205-0.880	0.021
	2015	1980	0.45	0.38	0.180-.819	0.013
Goat	2010	535	0.00	-	-	-
	2011	470	1.70	Ref	-	-
	2012	868	0.12	0.07	0.008-0.534	0.011
	2013	250	0.00	-	-	-
	2014	304	3.29	1.96	0.766-0.5.034	0.16
	2015	1115	0.54	0.31	0.108-0.905	0.032
All ruminant	2010	13866	0.63	Ref	-	-
	2011	14513	0.21	0.34	0.225-0.511	0.000
	2012	11700	0.31	0.49	0.331-0.721	0.000
	2013	12027	0.38	0.61	0.425-.870	0.006
	2014	18204	0.49	0.78	0.579-1.047	0.097
	2015	19280	0.55	0.88	0.665-1.174	0.394

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Table 7. Results of bacteriological analysis fortissue samples from serologically positive animals for brucellosis

Species \ Parts	Lymph nodes		Spleen		Total	
	Samples, n	+ve, n (%)	Samples, n	+ve, n (%)	Samples, n	+ve, n (%)
Cattle	23	9.0 (39.13)	4.0	2.0 (50)	27	11 (40.74)
Buffalo	15	6.0 (40)	2.0	1.0 (50)	17	7.0 (41.18)
Sheep	12	4.0 (33.33)	2.0	1.0 (50)	14	5.0 (41.67)
Goat	11	3.0 (27.27)	2.0	1.0 (50)	13	4.0 (30.77)
Total, n (%)	61	22 (36.07)	10	5.0 (50)	71	27 (38.03)

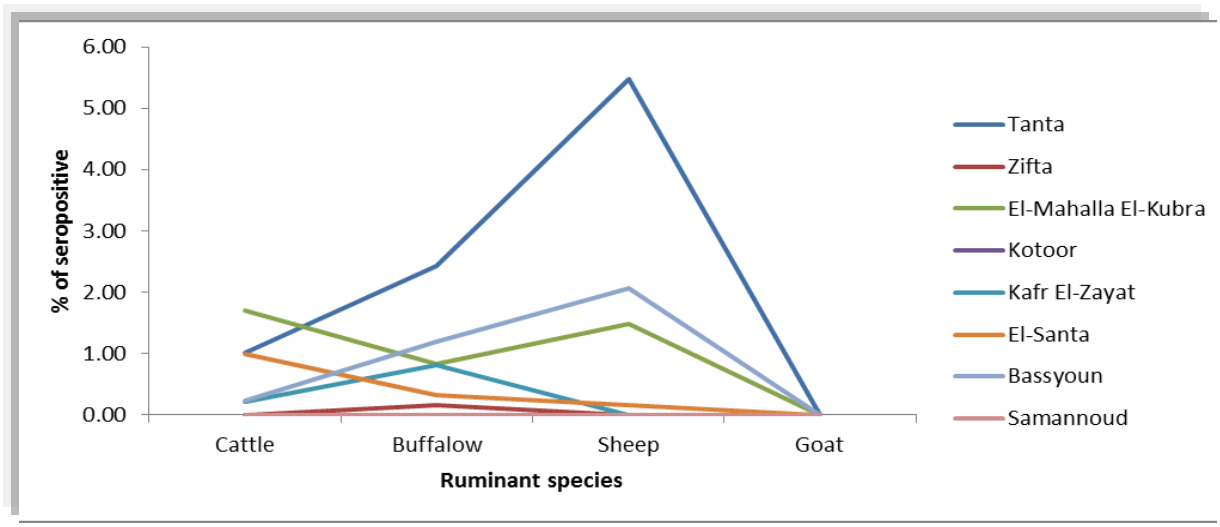

Figure 2. Distribution of seropositive ruminants per district in 2010 in Gharbia Governorate, Egypt

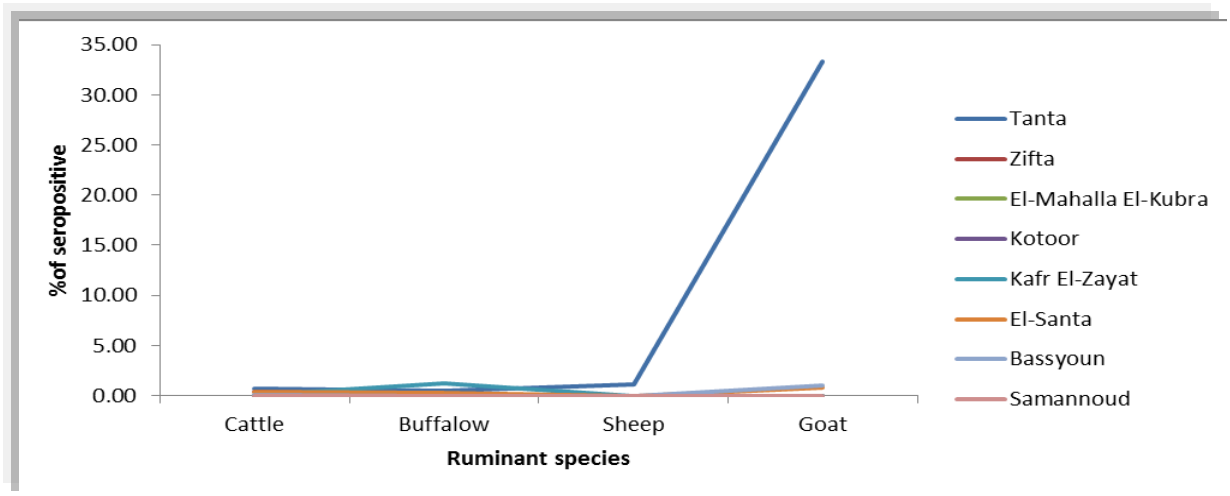

Figure 3. Distribution of seropositive ruminants per district in 2011 in Gharbia Governorate, Egypt

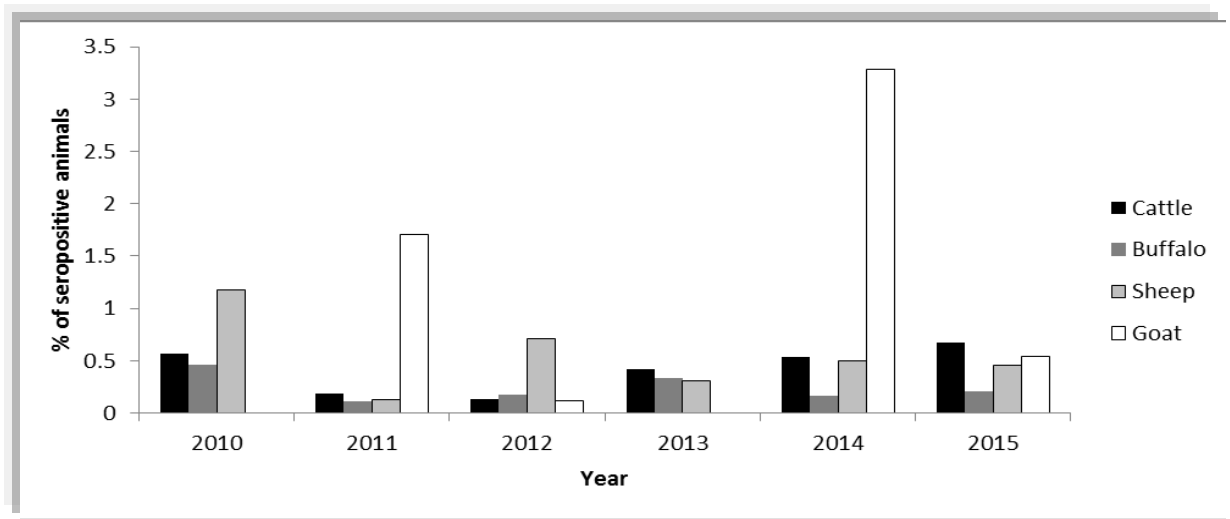

Figure 4. Seropositive ruminates for brucellosis in Gharbia Governorate, Egypt from 2010 to 2015

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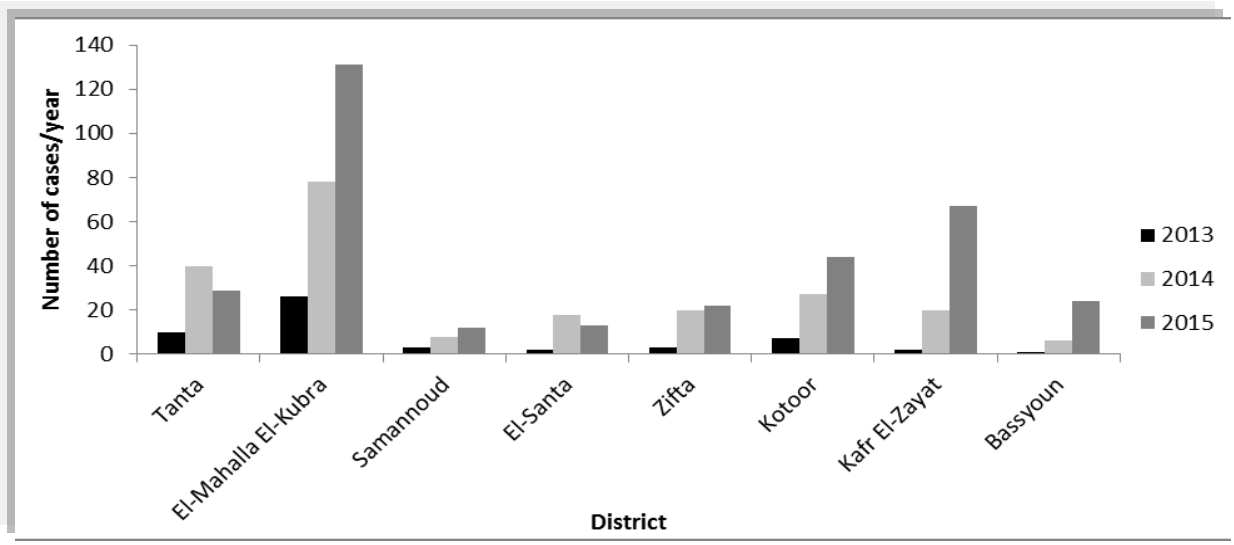

Figure 5. Reported human cases of brucellosis per district in Gharbia Governorate, Egypt from 2010 to 2015

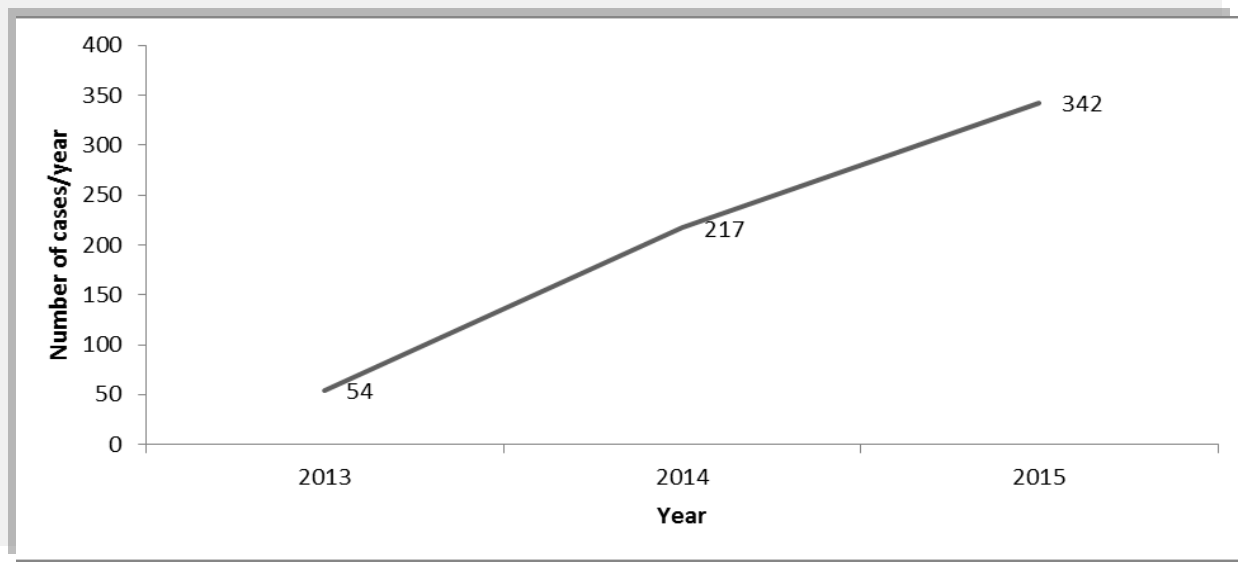

Figure 6. Reported human cases of brucellosis in Gharbia Governorate, Egypt from 2010 to 2015

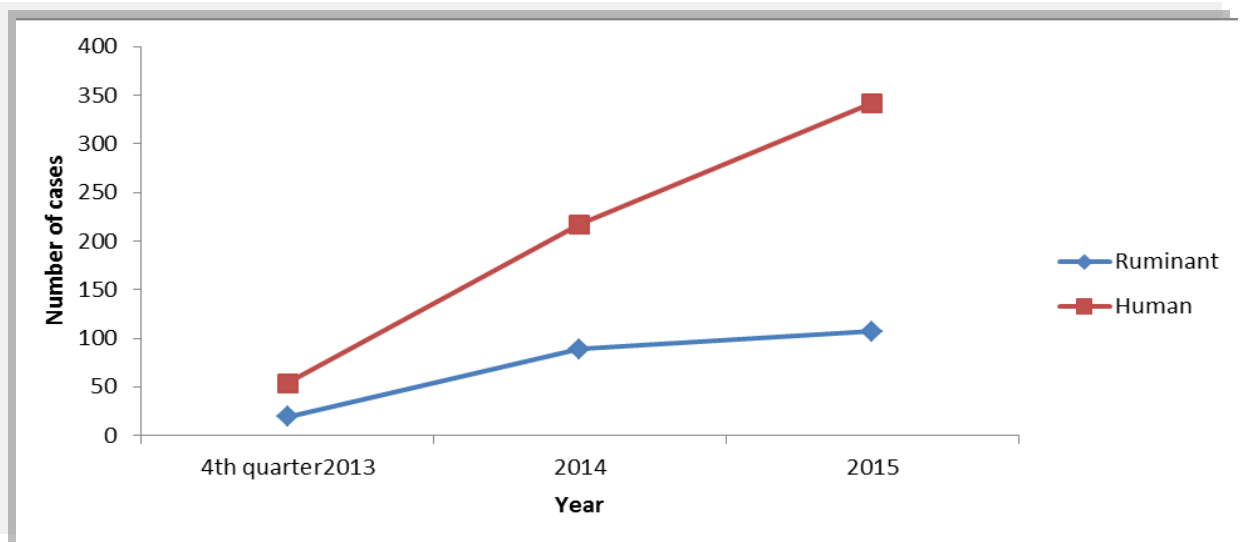

Figure 7. Reported animal in human cases of brucellosis in Gharbia Governorate, Egypt from September-2013 to December-2015

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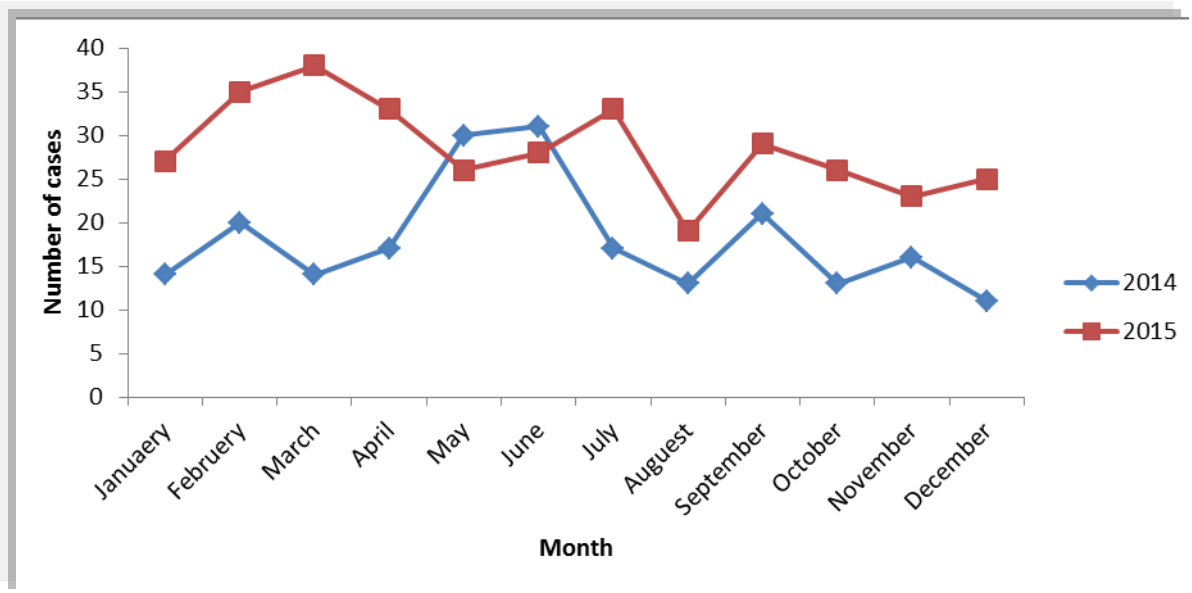

Figure 8. Monthly distribution of reported human cases of brucellosis in Gharbia Governorate for 2014 and 2015

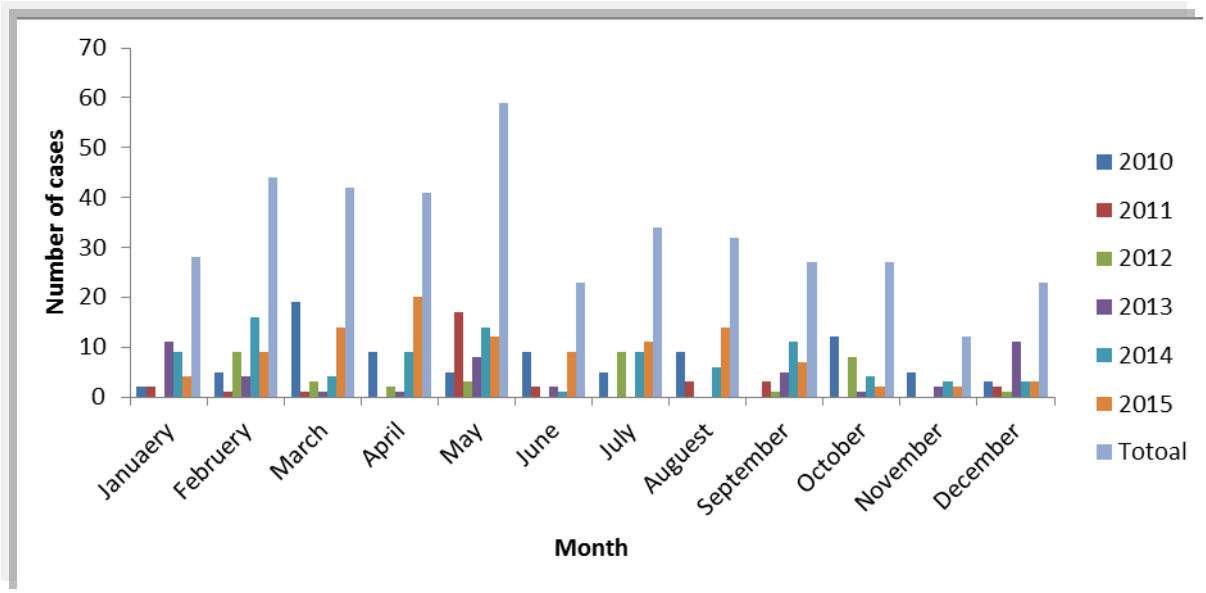

Figure 9. Monthly distribution of reported cases of brucellosis in ruminants in Gharbia Governorate from 2010to 2015

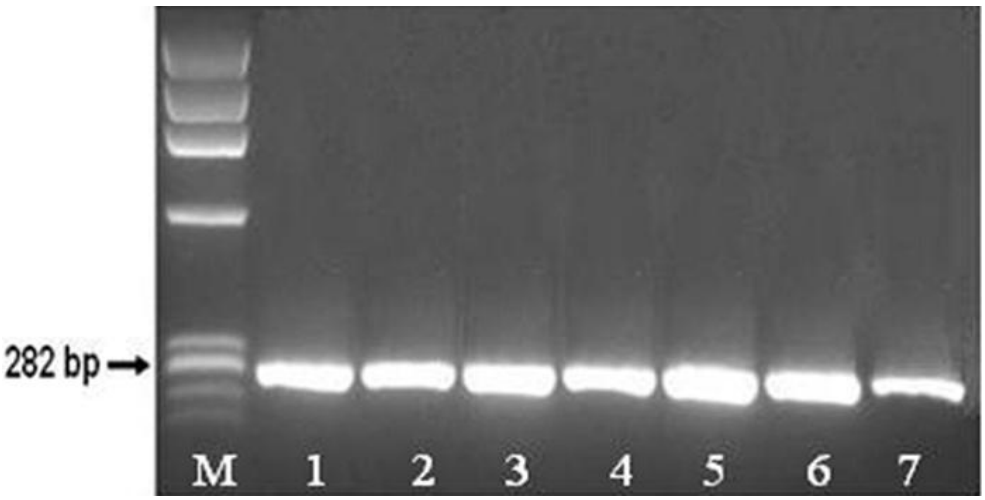

Figure 10. Agarose gel electrophoresis of PCR-amplified omp 2 gene fragments from *Brucellamelitensis* strains. The figure shows a single band 282-bp DNA fragment. M: ØX 174 RF DNA HaeIII digest marker (Biolabs). Lane, 1 *B. Melitensis*biovar 3 field strain. Lane

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DISCUSSION

The aim of this study was to assess the impact of the national brucellosis control programme on the temporal pattern of brucellosis in ruminants and human in Gharbia governorate, Egypt. The results indicated that in any given year of the study period (2010 to 2015), the proportion of tested ruminants for brucellosis was less than 3%. Cattle were the highest species to be frequently tested. There were no consistency in sampling of different ruminant species particularly goat and also there were no consistency in sampling in different districts of Gharbia governorate. These findings were similar to that in El-Behira governorate, where cattle were the highest species to be tested and for which 70% of the control programme costs were spent (Eltholth et al., 2015). Analysis of data from the brucellosis control programme in Kafrelsheikh governorate also indicated that, the fractions of ruminant sampled ranged from 2.2% to 6.5%, the percentages of sampling per species were 2.2% and 7.5% for cattle, 1.4% and 7.4%for buffaloes, 0.9% and 4.8% for sheep and 0.4%and 4.6% for goats(Hegazy et al., 2009). The results showed that the proportion of seropositive animals was low and there were variations in seropositive proportions in different species of ruminates and between districts. Given that there was no clear sampling strategy and no adherence to the programme, the results were not reliable to estimate the prevalence of the disease in the study area. For example negative results for goat in some districts for two consecutive years did not mean that goat in these districts were free from brucellosis; it was simply because goat was not sampled. Comparing with results from other studies in the same governorate and the neighbor ones, this proportion of seropositive animals was quite low (Holt et al., 2011; Hegazy et al., 2011; Mahboub et al., 2013 and Hegazy et al., 2016). A cross-sectional study was conducted in two villages in Gharbia governorate, in which the proportions of seropositive sera were 0.0 and 16% among livestock of villages I and II, respectively (El Sherbini et al., 2007). In El-Behira governorate, the proportion of seropositive cattle, buffalo, sheep and goats for brucellosis was found to be 5.86%, 5.83%, 7.20% and 11.33%, respectively(Sayour and Azzam, 2014). Results of regression analysis showed that, the proportion of seropositive ruminates fluctuated up and down. A significant increase in the proportion of seropositive animals after a significant reduction might be related to the variation in the number of tested animals for each year and non-adherence to the programme (Hegazy et al., 2009; Eltholth et al., 2015 and Eltholth et al., 2016). In our opinion if there was an actual reduction in the prevalence of the diseases due to the control programme, the proportion of seropositive animals would not have increased again unless there were other factors. The results of PCR indicated that all isolates were *B.melitensis* biovar 3 which thought to be the most common prevalent strain of brucellosis in Egypt (Refai, 2002; Montasser et al., 2012 and Ramadan et al., 2013).

The number of reported human cases of brucellosis was increasing from 2014 to 2015. This was positively associated with the increase of the proportion of seropositive ruminates in the same years. These results indicated that, controlling the disease in livestock would prevent human exposure to brucellosis. A previous study in the same governorate found that, keeping sheep in the household was a significant risk factor for human brucellosis (P=0.01) and among livestock, sheep showed the highest seropositive proportions of brucellosis(El Sherbini et al., 2007). The highest number of reported human cases in2014was in June and that for 2015 was in March. On the other hand the highest total number of reported cases per month in ruminants within the study period was May followed by February. These results indicated the association between brucellosis in animals and human and this time space could be the incubation period for infected human to develop symptoms. In another word, waves of cases in humans come after the increase of seropositive proportion in livestock. Furtherepidemiological studies should be conducted not only to assess the prevalence of disease in livestock and human but also to assess the risk factors of livestock infection for terms of prophylaxis.

Competing interests

The authors declare that there are not significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

REFERENCES

Alton GG, Jones LM, Angus R and Verger J (1988). Techniques for the brucellosis laboratory, Institut National de la recherche Agronomique (INRA).

Bardenstein S, Mandelboim M, Ficht TA, Baum M and Banai M (2002). Identification of the Brucella melitensis vaccine strain Rev. 1 in animals and humans in Israel by PCR analysis of the PstI site polymorphism of its omp2 gene. Journal of clinical microbiology, 40: 1475-1480.

Bricker BJ and Halling SM (1994). Differentiation of Brucella abortus bv. 1, 2, and 4, Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR. Journal of Clinical Microbiology, 32: 2660-2666.

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Journal homepage: www.wvj.science-line.com

El Sherbini A, Kabbash I, Schelling E, El Shennawy S, Shalapy N, Elnaby GH, Helmy AA and 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: 923-928.

Eltholth M, Hegazy Y, El-Tras W, Bruce M and Rushton J (2016). Temporal Analysis and Costs of Ruminant Brucellosis Control Programme in Egypt Between 1999 and 2011. Transboundary and Emerging Diseases, DOI: 10.1111/tbed.12491.

Eltholth Mm, El-Wahab EWA, Hegazy YM and El-Tras WF (2015). Assessing Impacts and Costs of Brucellosis Control Programme in an Endemic Area of the Nile Delta, Egypt. World's veterinary journal, 5: 74-81.

Gwida M El-Ashker, M El-Diasty M, Melzer F andNeubauer H (2015). Seroprevelance of Bovine Brucellosis in the Nile Delta Region, Egypt: APreliminary Study. J Vet Med Res 2(5): 1037.

Hassanain N and Ahmed W (2012). Sero-prevalence of brucellosis in Egypt with emphasis on potential risk factors. World Journal of Medical Sciences, 7: 81-86.

Hegazy Y, Elmonir W, Abdel-Hamid NH and Elbauomy EM (2016). Seroprevalence and "Knowledge, Attitudes and Practices"(KAPs) survey of endemic ovine brucellosis in Egypt. Acta Veterinaria Scandinavica, 58: 1.

Hegazy Y, Ridler A and Guitian F (2009). Assessment and simulation of the implementation of brucellosis control programme in an endemic area of the Middle East. Epidemiology and infection, 137: 1436-1448.

Hegazy Y, Moawad A, Osman S, Ridler A. and Guitian J (2011). Ruminant brucellosis in the Kafr El Sheikh Governorate of the Nile Delta, Egypt: prevalence of a neglected zoonosis. PLoS neglected tropical diseases, 5: e944.

Holt HR, Eltholth MM, Hegazy YM, El-Tras WF, Tayel AA and Guitian J (2011). Brucella spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC public health, 11: 341.

Kaoud H Zaki Mm, Shimaa A and Nasr A (2010). Epidemiology of brucellosis among farm animals. Nature and Science, 8: 190-197.

Mahboub HD, Helal MA, Eldaim A Attia M, El-Razek A, Mahmoud E And Elsify AM (2013). Seroprevalence of Abortion Causing Agents in Egyptian Sheep and Goat Breeds and Their Effects on the Animal's Performance. Journal of Agricultural Science (1916-9752), 5.

Mcdermott J Grace D and Zinsstag J (2013). Economics of brucellosis impact and control in low-income countries. Revue scientifique et technique (International Office of Epizootics), 32: 249-261.

Montasser M, Khoudair M Soliman S and Eman AK (2012). Evaluation of immunochromatographic assay for serodiagnosis of Brucella among cattle, sheep and goats in Egypt. Global Veterinaria, 8: 511-518.

Pappas G and Memish Z (2007). Brucellosis in the Middle East: a persistent medical, socioeconomic and political issue. Journal of Chemotherapy, 19: 243-248.

Ramadan KM, Hazem SS and Khairy EA (2013). Seroprevalence of Brucella Infection among Buffaloes in Gharbyia Governorate. population, 2: 8.

Refai M (2002). Incidence and control of brucellosis in the Near East region. Veterinary microbiology, 90, 81-110.

Samaha H, Al-Rowaily M, Khoudair RM and Ashour HM(2008). Multicenter study of brucellosis in Egypt. Emerging infectious diseases, 14: 1916.

Sayour AE and Azzam RA (2014). Epidemiological characterization and pathogenicity of prevalent Brucella strains in Egypt.Annals of Veterinary and Animal Science, eISSN: 2313-5514: 77-85.

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