Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 61 (1): 22-31. April 2019
DOI: 10.5455/ajvs.39738

Epidemiological and Zoonotic Surveillance of Brucellosis in Beni-Suef Governorate

Eman S. Ramadan[1*], Nader R. Nassar[2], Ibrahim G. Ibrahim[3], Amany F. Zayed[4]
[1]Animal Reproductive Diseases Department, Animal Reproduction Research Institute, Giza, Egypt.
[2]Field Tests Department, Animal Reproduction Research Institute, Giza, Egypt.
[3]Director, Animal Reproduction Research Institute, Giza, Egypt.
[4]Food Control Department, Animal Health Research Institute, Alexandria, Egypt.

Key words:
BAPAT, Brucellosis, CFT, Egypt, RBPT, Risk factor

***Corresponding to:**
Emanramadan1311971@gmail.com

Article History
Received 01 Mar 2019
Revised 30 Mar 2019
Accepted 30 Mar 2019

ABSTRACT

A cross-sectional study was carried out in a village in Beni-Suef Governorate in the Upper Egypt from August 2018 till February 2019. A total of 1121 animals (574 cows and 547 buffaloes) and 600 human serum samples were examined for brucellosis using buffered acidified plate antigen test (BAPAT), rose Bengal plate test (RBPT) and Complement fixation test (CFT). Statistical analysis was applied using Chi-square and IBM® SPSS statistic. The results of BAPAT, RBPT, and CFT were 8.71%, 8.36%, and 8.36% respectively in examined cows samples and were 8.23%, 7.86%, and 7.68% respectively in examined buffaloes samples with Odds ratio (OR) of 1.067, 1.071, and 1.096 respectively showing the same chance and susceptibility of both species to be infected. Where human sera gave 6.5%, 6.3%, and 6.3%, respectively. Considering the human occupation, it was with highly significant value (Chi-square=49.23 and P=0.001) representing a risk factor with the most predominant of butchers and abattoir workers who gave 22.22% positives with Odds of infection=0.286 for all used tests. Followed by veterinarians & veterinary attendants and farmers & householders (14.28% and 10.24%) positives with Odds of infection (0.167 and 0.114) respectively for all used tests. In relation to the age groups showed a significant value (Chi-square=9.69 and P=0.046) for all used tests expressing that the age acted as a risk factor. Ages of (36-45 years) appeared to be the most group at the risk (Odds of infection=0.1206) than younger or/and older ones. Conclusively, brucellosis is endemic at high levels among the large ruminants in Beni-Suef Governorate in the Upper Egypt. There was an urgent need for implementing a proper control program for bovine brucellosis and more attention should be paid towards improving the animal health delivery system in those governorates that are large in size and share borders with other countries. The need for reinforcement of the integrated "One Health" approach. Further studies on the causative agent isolation and identification should be needed.

1. INTRODUCTION

Brucellosis, also known as “Malta fever”, “Mediterranean fever” or “undulant fever” is still the most important occupational disease of veterinarians and those involved in farm animal production (Bricker, 2002). In the Mediterranean and Middle East countries *Brucella* infection causes severe problems giving rise to major economic losses in livestock breeders through interference with the breeding programs by decreasing in the reproductive efficiency, abortion, decrease in milk yield and also serious impact on public health (Hassan and Samaha, 2008). The dairy animals e.g. cattle, sheep, goat, and camels are included within the reservoirs of the *Brucella* agent (Adams and Moss, 1995). Brucellosis is one of the major zoonotic diseases. The first reported predominate species of *Brucella* in cattle and buffaloes in Egypt was *Brucella*

(B.) melitensis in 1939 (Refai, 2002; Hegazy et al., 2009). In the dairy animals, *Brucella*e centralize in the supramammary lymph nodes which continue to excrete them in the milk (Cordes and Carter; 1979; Refai, 2003).

Human brucellosis mostly occurs among farmers, butchers and veterinarians according to their close contact with animals, contaminated fetal membranes, infected animals, infected materials, and also human may get infected through ingestion of infected animal products (Wallach et al., 1994) like as raw liver, half-baked condition or consuming raw milk and non-pasteurized dairy like milk, butter, cream and cheese (Lindahl et al., 2015). Brucellosis in man is considered as a predisposing factor leading to infertility and sterility. Treatment of this disease is done with the goal of elimination of the illness symptoms and decreasing the complications like arthritis, miscarriage, spondylitis, and stopping the relapse of the infection (del Pozo and Solera, 2012; Smailnejad et al., 2012).

Serological tests have been used extensively throughout the world for the diagnosis of brucellosis in man and animals, the Rose Bengal Plate Test (RBPT) and Buffered Acidified Plate Antigen (BAPAT) are of choice in the diagnosis and to minimize the risk of errors (Morgan et al., 1969; Alton et al., 1975). On the other hand, Plumeriastuti and Zamri-Saad, (2012) reported that the usage of RBPT for the screening of infected herd followed by the complement fixation test (CFT) on RBPT positive sera leads to the culling of many infected breeders.

Proper diagnosis of infected animals considered the first priority in control programmes of brucellosis as early as possible in a herd and this is considered a pre-request for the successful control and elimination of brucellosis. It is difficult to use a control programme for brucellosis in Egypt (Refai, 2002; Hegazy et al., 2009).

The aim of the current work was to investigate the epidemiological and zoonotic surveillance of brucellosis in Beni-Suef Governorate supposing that keeping cattle and buffaloes in a mixed population with sheep or/and goats in the same household would be a risk factor for brucellosis, as these species are the primary hosts of *B. melitensis*, which is presently the predominant species of *Brucella* in Egypt. The study objectives were; estimation of seroprevalence of *Brucella* spp. in cattle and buffaloes at the individual animal and household level, and identification the risk factors for both species, and assessment the risk factors of in contact humans regarding their occupations and ages.

2. MATERIAL AND METHODS

2.1. Sampling plan:

This study was conducted in a village in Beni-Suef Governorate in the Upper Egypt. It was conducted by a cross-sectional study. All samples were tested in the laboratories of the Animal Reproduction Research Institute- Giza -Egypt. As there was no sampling frame in the village, the target sample size for simple random sampling was calculated to be 300 houses as the total houses of the village were estimated to be about 3000 households, so a sampling interval of 10 was used.

One road leading away from the village market, which is the center of the village, was selected randomly, along this road one household was selected randomly to be the point of the beginning of the subsequent systematic selection of houses, every 10 houses along this road and all side streets leading from it, was selected. Once the outskirts of the village were reached sampling continued clockwise until another road was reached, this road was taken back towards the center of the village which continually sampling houses, next the road opposite the first road sampled was taken, and the sampling continued in this manner.

In each selected house, the householder was asked if any cows, buffaloes, sheep or goat were kept in the house, and he was asked also for oral agreement to take him and his wife as a volunteer in brucellosis study.

During the initial visit, (from August 2018 till February 2019) from every household blood samples were taken from any person who is responsible for milking the animal or animal caretaker and its animals (cows and buffaloes). The structure designed a questionnaire to know the Occupation and socio-demographic characters of householders.

2.2. Animal blood samples:

Blood samples were taken from 1121 animals (574 cows and 547 buffaloes) aseptically by vein puncture where the skin over the Jugular vein was prepared by clipping, defatted by rubbing with a swab soaked in alcohol, then disinfected by tincture iodine. About 10ml of blood was aseptically drawn from jugular vein into a sterile scew capped Mac-Carteny bottle. The bottles were left in room temperature in a slopping position to allow clot formation. The collected samples were marked, identified and transferred to the laboratory, where they were held in the refrigerator till the next day to give a chance for the serum to separate.

The clear serum was siphoned off and stored at 2-8 ℃ for 48hrs in the refrigerator till use if they are to be stored longer, they should be frozen. (Alton et al., 1988)

2.3. Human blood samples

Human blood samples were collected from 600 humans (males and females) from brachial vein (WHO, 1989) after disinfection of the vein area with alcohol, using disposable sterile syringes and needles. The obtained blood was transferred into sterile dry Mac-Carteny bottles and serum was collected as mentioned before.

2.4. Serological testes

2.4.1. Buffered acidified plate antigen test (BAPAT)

All the examined 1121 cows and buffaloes' serum samples were tested using buffered acidified plate antigen (BAPA) provided by Veterinary Serum and Vaccines Research Institute (VSVRI) (Abbasia Laboratories, Abbasia, Cairo, Egypt). The six hundred human serum samples were tested using cromatest which was obtained by (LiNEAR, Barcelona, Spain). Any degree of agglutination was considered positive results. (OIE, 2015).

2.4.2. Rose Bengal Plate Test (RBPT)

All tested serum samples (574 cows, 547 buffaloes, and 600 humans) were examined using antigen stained with rose Bengal and buffered to a low pH, 3.65 ± 0.05 (IDEXX Laboratories, Pourquier, Hoofddorp, the Netherlands) any degree of agglutination was considered as positive results. The serum samples and antigen were carried at room temperature (22°C ± 4°C). (OIE, 2016).

2.4.3. Complement fixation test (CFT)

Positive RBPT serum samples were retested for anti-*Brucella* antibodies with CFT validated for the detection of anti-*Brucella* antibodies in cattle (Brucellosis CFT) provided by (Jovac, Jordan Bio Industries center, Amman, Jordan) which contained all the necessary reagents. The test was performed according to the manual which is accompanied with the kit (OIE, 2016).

2.5. Statistical analysis:

Chi-square statistic was used and ($p<0.05$) using IBM® SPSS statistic version 20 (SPSS Inc., Chicago, Illinois, USA).

NS: Non-significant ($P > 0.05$).

OR = Odds Ratio, which is considered as a measure of association used to quantify the relative risk of one category to another.

Interpretation of the Odds Ratio (OR):

The higher the odds, the higher the risk of such category to disease occurrence.

OR = 1: The exposure (risk factor) is not associated with outcome or disease.

(Or, No association between the disease and risk factor).

OR > 1: Increased exposure (risk factor) accompanies increased outcome or disease. (Or, a positive association between the disease and risk factor).

OR < 1: Increased exposure (risk factor) accompanies decreased outcome or disease. (Or, a negative association between the disease and risk factor).

3. RESULTS AND DISCUSSION

The current study revealed that the seroprevalence of brucellosis using BAPAT, RBPT, and CFT as shown in Table (1) was 8.71%, 8.36%, and 8.36% respectively in cows and was 8.23%, 7.86%, and 7.68% respectively in buffaloes**.** Higher results were recorded by Holt et al. (2011) 11.0% for cattle and 15.5% for buffaloes. However, our results were higher than those obtained previously by Samaha et al. (2008), who estimated an overall seroprevalence of 5.44% in cattle and 4.11% in buffaloes, confirming that brucellosis is endemic in the studied village. Although animals were tested for antibodies against *Brucella* spp., recovery from *Brucella* infection in ruminants is rare and they usually remain infected for life and acted as a chronic carrier (Crawford, 1990). Although eradication of cattle and small ruminants in a handful of industrialized countries, brucellosis remains endemic in most areas of the world (Moreno, 2014). Also, as an abortion in cows or buffaloes is likely to have a greater economic impact on the household than in sheep or goats, there could be more awareness of the disease events in large ruminants (Corbel, 1988). In our study, the organisms infected large ruminants were not isolated and typed, however, *B. melitensis* is the predominant *Brucella* species in Egypt (Ramadan and Ibrahim, 2014; Ramadan and Gafer, 2016). *B. melitensis* mainly causes abortions, stillbirths, the birth of weak offspring and sometimes retained placenta. Sheep and goats usually abort only once, but reinvasion of the uterus and shedding of organisms can occur during subsequent pregnancies. In animals that abort as well as those whose udder becomes infected after a normal birth, milk yield is markedly reduced. However, clinical signs of mastitis are uncommon. Acute orchitis and epididymitis may occur in males and can result in infertility. Arthritis is seen occasionally in both sexes (DelVecchio et al., 2002).

Table (1) Serological tests of brucellosis in examined serum samples collected from cows and buffaloes (n=1121).

Animal species	No. of examined serum samples	BAPAT		RBPT		CFT	
		Positives	%	Positives	%	Positives	%
Cows	574	50	8.71%	48	8.36%	48	8.36%
Buffaloes	547	45	8.23%	43	7.86%	42	7.68%
Total	1121	95	8.47%	91	8.12%	90	8.02%

BAPAT: Buffered acidified plate antigen test. RBPT: Rose Bengal plate test. CFT: Complement fixation test.

Table (2) Risk factors associated with brucellosis serological status in examined serum samples collected from cows and buffaloes (n=1121)

Serological test	No. of examined samples	P - value	OR (cows vs buffaloes)	Chi-square value	df	Odds of infection (Positive /Negative)	
						Cows	Buffaloes
BAPAT	1121	0.794	1.067	0.677^{NS}	1	0.095	0.089
RBPT	1121	0.982	1.071	0.081^{NS}	1	0.091	0.085
CFT	1121	0.992	1.096	0.094^{NS}	1	0.091	0.083

BAPAT: Buffered acidified plate antigen test. RBPT: Rose Bengal plate test. Complement fixation test.
OR: Odds ratio NS: Non-significant (P > 0.05). df: Degree of freedom

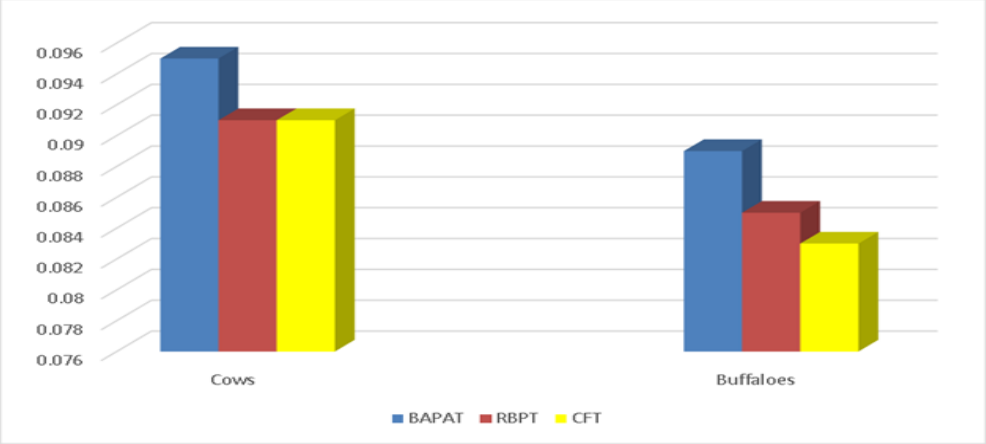

Fig. (1) Risk factors associated with brucellosis serological status in examined serum samples collected from cows and buffaloes (n=1121)

Table (3) Serological tests of brucellosis in examined serum samples collected from human (n=600).

No. of examined serum samples	BAPAT		RBPT		CFT		Chi-square	P- value	df
	Positives	%	Positives	%	Positives	%			
600	39	6.5%	38	6.3%	38	6.3%	0.019^{NS}	0.991	2

BAPAT: Buffered acidified plate antigen test. RBPT: Rose Bengal plate test. CFT: Complement fixation test.
OR: Odds ratio. NS: Non-significant (P > 0.05). df: Degree of freedom.

Table (4) Occurrence of positive Serological tests of brucellosis reactors in relation to their occupation from examined serum from human (n=600)

Occupation	No. of examind samples	BAPAT			RBPT			CFT		
		Positives	%	Odds of infection	Positives	%	Odds of infection	Positives	%	Odds of infection
Farmers & householders	127	13	10.24	0.114	13	10.24	0.114	13	10.24	0.114
Milker's housewives	234	16	6.83	0.073	15	6.41	0.068	15	6.41	0.068
Non-milker's housewives	66	1	1.52	0.015	1	1.52	0.015	1	1.52	0.015
Students	24	1	4.17	0.043	1	4.17	0.043	1	4.17	0.043
Agricultural workers	65	3	4.62	0.048	3	4.62	0.048	3	4.62	0.048
Butchers & abattoir workers	9	2	22.22	0.286	2	22.22	0.286	2	22.22	0.286
Vets. & Vet. Attendants	7	1	14.29	0.167	1	14.29	0.167	1	14.29	0.167
Another occupations	68	2	2.94	0.030	2	2.94	0.030	2	2.94	0.030
Total	600	39	6.50	---	38	6.33	---	38	6.33	---
Chi-square	600		49.23**			49.23**			49.23**	
P – value	600		0.001			0.001			0.001	

BAPAT: Buffered acidified plate antigen test. RBPT: Rose Bengal plate test. CFT: Complement fixation test. **: Highly significant (P < 0.01)

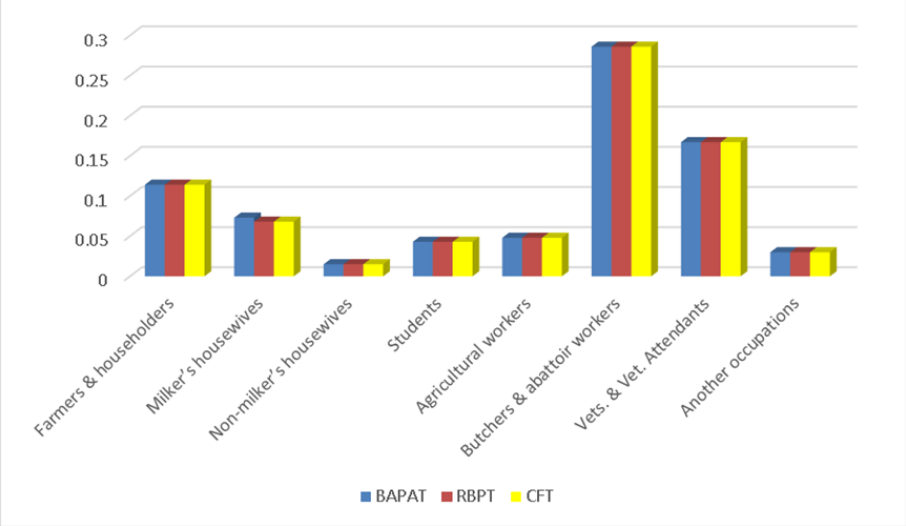

Fig. (2). Odds of infection with human brucellosis reactors in relation to their occupation (n=600)

Table (5) Occurrence of positive brucellosis serological tests reactors in relation to age groups from examined human serum (n=600)

Age group	No. of examined samples	BAPAT			RBPT			CFT		
		Positives	%	Odds of infection	Positives	%	Odds of infection	Positives	%	Odds of infection
16-25 years	17	1	5.88%	0.0625	1	5.88%	0.0625	1	5.88%	0.0625
26-35 years	96	6	6.26%	0.0667	6	6.25%	0.0667	6	0.0667	0.067
36-45 years	223	24	10.76%	0.1206	23	10.31%	0.1150	23	10.31%	0.1150
46-55 years	195	7	3.59%	0.0372	7	3.59%	0.0372	7	3.59%	0.0372
More than 55 years	69	1	1.45%	0.0147	1	1.45%	0.0147	1	1.45%	0.0147
Total	600	39	6.5%	---	38	6.33%	---	38	6.33%	----
Chi-square	600		9.69*			9.69*			9.69*	
P – value	600		0.046			0.046			0.046	

BAPAT: Buffered acidified plate antigen test. RBPT: Rose Bengal plate test. CFT: Complement fixation test. **: Significant (P < 0.05)

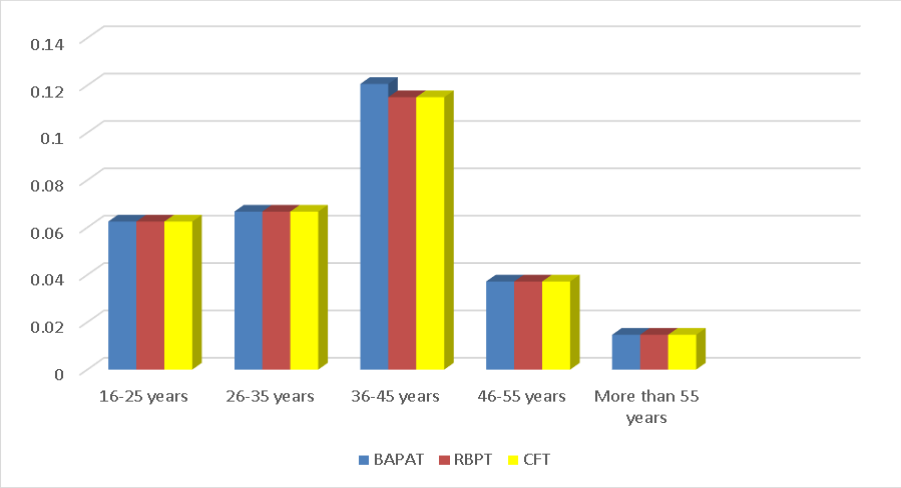

Fig. (3). Odds of infection with human brucellosis reactors in relation to their ages (n=600)

Regarding the risk factors associated with brucellosis serological status in examined serum samples collected from cows and buffaloes as shown in Table (2) and Fig. (1), we should not expect to find a significant difference between studied groups (P > 0.05). Results showed that species is not a risk factor. Therefore, both species have the same chance and susceptibility to being infected with brucellosis. These results were in the same context with other studies reported by Samaha et al., (2008) who found the seroprevalence of brucellosis was 4.98 % for cattle and 3.52% for buffaloes (nearly the same percent) reporting the highest prevalence in Beni-Suef governorate than other areas of Egypt. The mixed population of large ruminants with sheep and goats in the household was the primary risk factor for cattle and buffaloes testing serologically positive for *Brucella* spp., which was coincided with other studies in areas where *B.*

melitensis is the predominant species present, therefore it appeared that seropositive cattle and buffaloes were infected with the same species (Omer et al., 2000; Samaha et al., 2008). The current study focused on brucellosis in large ruminants and no sheep or goats were tested in the village. A recent study, in 40 villages from nearby Kafr Al-Sheikh Governorate measured that the village flock of small ruminants was infected in more than 60% of villages with Seroprevalence of 13.5% and 12.5% in sheep and goats respectively. These village flocks were mobile and used for breeding with household animals (Hegazy et al., 2011). Our finding in large ruminants declared that *Brucella* spp. was likely to be present in small ruminants in the village, probably at a high level (Holt et al., 2011). More than 70% of Egyptian total livestock was owned by small householders in a few numbers of cows and buffaloes (Aidaros, 2005).

On the other side, serological tests of brucellosis in examined serum samples collected from human were shown in Table (3). The incidences of *Brucella* antibodies in a total of 600 random serum samples collected from human by using BAPAT, RBPT, and CFT were 6.5%, 6.3%, and 6.3%, respectively. Furthermore, the differences associated with such examined samples were non-significant ($P > 0.05$) as a result of the used serological test for brucellosis. Human behavior may influence the spread of the disease, both between animals and from animals to humans. Brucellosis happens naturally in domestic animals and it may be carried to human beings by direct or indirect tools such as close contact with infected livestock, their secretions or tissues, herding, lambing, and consumption of unpasteurized milk and dairy products (Alballa, 1995; HusseiniAS and Ramlawi, 2004). Placentas and aborted fetuses were disposed of by most people into water canals, which acted as a source of infection because most animals in this area might access the water canals for drinking and bathing. *B. melitensis* was previously isolated from catfish (El-Taras et al., 2010). Moreover, *B. melitensis* biotype 3 which is the most common isolate of *Brucella* spp. in Egypt was isolated from rats (Ramadan and Ibrahim, 2014). Rats were often found near canals and dogs might use these canals for bathing or drinking, therefore these species acted as a thread aiding the spread of brucellosis (Azzam et al., 2009).

Data presented in Table (4) and Fig. (2) revealed that the occurrence of positive serological reactors of brucellosis in relation to their occupation was highly significant (Chi-square=49.23 and P=0.001) for all used tests representing a risk factor.

Butchers and abattoir workers gave the highest percentage (22.22%) with Odds of infection=0.286 for all three used tests representing the most predominant risk factor among other occupations. Followed by veterinarians & veterinary attendants and farmers & householders (14.29% and 10.24%) with Odds of infection (0.167 and 0.114) respectively for all used tests. These findings were coincided by (Soliman, 1998; Saleh et al., 2003; Manal, 2011). On the contrary, Yoo et al., (2009); Acharya et al., (2018) showed lower Seroprevalence (6.1% and 6.7%) respectively among Korean slaughterhouse workers.

The highest human brucellosis seroprevalence reported for butchers and abattoir workers might be attributed to poor educational level and length of service which made them did not wear protective equipment at work. Recent reports found that the butchers and butchers attendants who handled fetuses and uterine contents were more at risk compared to the other occupations probably due to their close contacts with infected tissues and blood of infected animals. (Awah-Ndukum et al., 2018).

Other many reports in Tanzania, Nigeria, and Egypt highlighted that among occupational groups in abattoirs, seroprevalence of brucellosis was highest among butchers whose main job was slaughtering of animals, followed by livestock traders, meat sellers and abattoir cleaners compared with the other workers (Cadmus et al., 2006; El Kholy et al., 2009; Swai and Schoonman, 2009; Aworh et al., 2013). Moreover, transmission of human brucellosis by inoculation through abrasions and cuts in the skin (Young, 1995; Corbel, 2006). Veterinarians could contact the disease in removing the retained placenta, animal discharges, treating animals, laboratory culturing and handling living vaccine (Corbel, 2006).

Farmers and householders gave 13(10.24%) seropositives, and milker's housewives gave 15(6.41%). However, the lowest seroprevalence was obtained in agricultural workers, students and non-milker's housewives "3 (4.62%), 1(4.17% and 1(1.52%)" respectively. This finding coincided with other previous reports showing that in the process of transmission of brucellosis to susceptible human hosts, direct contact with infected animals and their products was more important than the ingestion of contaminated animal products (Sofian et al., 2008; Nematollahia et al., 2017). Some farmers if they believed that their animals were infected with *Brucella* spp, they preferred

to sell them in markets than to notify the veterinary authorities and wait until they test and slaughter the positive animals. Consequently, this could increase the transmission of brucellosis, not only between households in the same village but also between villages and even larger geographical areas because the purchased animals could be moved without restriction to anywhere in Egypt (Holt et al., 2011).

However, there was a potential risk of exposure from other dairy products processed and consumed regularly in more than 80% of households. In the Egyptian governorate of Alexandria, *Brucella melitensis* has been isolated from raw cattle and buffalo's milk sold in dairy shops (Zayed et al., 2017). A hospital-based case-control study in Yemen showed substantial risk factors for infection related to occupation and drinking fresh milk. Additionally, socioeconomic and educational factors were also independent risk factors (Al-Shamahy and Wright, 2001).

Higher rates of brucellosis were observed among males than females. This finding was consistent with other reports who stated that the fact that brucellosis was mainly an occupational disease (abattoirs and veterinarians) might be another cause why the prevalence was higher among males than females (Aloufi et al., 2016).

The results of positive human brucellosis reactors in relation to their ages showed a significant value (Chi-square=9.69 and P=0.046) for all used tests as illustrated in Table (5) and Fig. (3) expressing that the age acted as a risk factor. Humans aged between 36 and 45 years were in a ratio of 10.76% and Odds of infection=0.1206 appeared to be the most group at the risk of infection than younger or/and older ones. FAO, (2014) reported that most cases of human brucellosis could occur during the spring and summer seasons among those aged 20–45 years. In addition to Aloufi et al., (2016) found that the highest risk group was aged (15-44) years. From the represented results, it was remarked that as the age was lower or/and higher than that range (36-45) years, the risk factor of infection would be decreased. That might be attributed to that other groups were less like to come into contact with infected animals (Aloufi et al., 2016). Additionally, brucellosis cases presented mainly with bouts of fever and musculoskeletal pain. As the nonspecific manifestation of brucellosis diagnosis, it might lead to underestimation and underreporting of brucellosis cases (Franco et al., 2007).

4. CONCLUSIONS

The current study proved that brucellosis is endemic at high levels among the large ruminants in Beni-Suef Governorate in the Upper Egypt, where the main risk factor for cattle and buffaloes was the presence of a mixed population with sheep or/and goats in the same household. There is an urgent need for implementing a proper control program for bovine brucellosis and more attention should be paid towards improving the animal health delivery system in those governorates that are large in size and share borders with other countries. The need for reinforcement of the integrated "One Health" approach which could be achieved through: 1) Isolation between diseased animals and other healthy ones. 2) The non-effective 'test-and-slaughter' policy for many years of implementation required a sound review of the brucellosis control policy. 3) A regular and frequent investigation should be applied for susceptible livestock, especially those in potentially epidemic areas. 4) Enforcing obligatory vaccinations of all susceptible animals. 5) Increasing the level of education and awareness among people, especially people who are at risk. 6) Pasteurization of milk and dairy products and education regarding eating habits must be followed, especially in rural areas. 7) In contact persons who may be at risk must be encouraged to consistently use personal protective equipment and good personal hygiene practices at work, regular brucellosis screening and adhering to safe animal-product handling practices. If it was difficult because of affordability and availability issues, other simple and cheap material such as plastic bags that are easily available in the rural areas. 8) Building collaborations between veterinarians, medical personnel, and other environmental authorities. Further studies on the causative agent isolation and identification should be needed.

5. ACKNOWLEDGMENTS

The authors would like to thank all staff of Directorate of veterinary medicine, veterinarians and assistants in Beni-Suef for their intimate cooperation in collecting the animal blood samples. Also, we are greatly thankful to doctors and nurses of Health Insurance clinic in Beni-Suef for their helpful aids for drawing the human blood samples. Finally, we are grateful to the animal owners for their helpful cooperation for completing this study.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

6. REFERENCES

Acharya, D., Hwang, S., Park, J. 2018. Seroreactivity and Risk Factors Associated with Human Brucellosis among Cattle Slaughterhouse Workers in South Korea. Int. J. Environ. Res. Public Health. 15(11): 2396.

Adams, M.R., Moss, M.O. 1995. Food microbiology. The Royal Society of Chemistry, Cambridge.

Aidaros, H. 2005. Global perspectives - the Middle East: Egypt. Rev. sci. tech. int. Epiz. 24(2):589–596.

Alballa, S.R. 1995. Epidemiology of human brucellosis in southern Saudi Arabia. J. Trop. Med. Hyg. 98:185–189.

Aloufi, A.D., Memish, Z.A., Assiri, A.M., McNabb, S.J. 2016. Trends of reported human cases of brucellosis, Kingdom of Saudi Arabia, 2004–2012. J. Epidemiol. Glob. Health. 6 (1): 11–18.

Al-Shamahy, H.A., Wright, S.G. 2001. A study of 235 cases of human brucellosis in Sana'a, Republic of Yemen. East. Mediterr. Health J. 7: 238–246.

Alton, G., Jones, L. M., Angus, R. D. Verger, J. M. 1988. Techniques for the Brucellosis Laboratory, INRA Publications, Paris,

Alton, G., Jones, L.M., Pietz, D.E. 1975. Laboratory techniques in brucellosis.2nd ed. FAO/WHO, Geneva.

Awah-Ndukum, J., Mouiche, M.M., Kouonmo-Ngnoyum, L., Bayang, H.N., Manchang, T.K., Poueme, R.S., Kouamo, J., Ngu-Ngwa, V., Assana, E., Feussom, K.J., Zoli, A. 2018. Seroprevalence and risk factors of brucellosis among slaughtered indigenous cattle, abattoir personnel and pregnant women in Ngaoundéré, Cameroon. BMC Infect. Dis. 18(1): 611.

Aworh, M.K., Okolocha, E., Kwaga, J., Fasina, F., Lazarus, D., Suleman, I., Poggensee, G., Nguku, P., Nsubuga, P. 2013. Human brucellosis: seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria - 2011. Pan. Afr. Med. J. 16:103.

Azzam, R.A., El-Gamal, A.M., Elsheemy, M.T. 2009. Failure of control of *Brucella melitensis* infection in a dairy herd. Assiut Vet. Med. J. 55(121):274–285.

Bricker, B.J. 2002. PCR as a diagnostic tool for brucellosis. Vet. Microbiol. 90:435–446.

Cadmus, S.I.B., Ijagbone, I.F., Oputa, H.E., Adesokan, H.K., Stack, J.A. 2006. Serological survey of brucellosis in livestock animals and Workers in Ibadan, Nigeria. African J. Biomed. Res. 9:163–168.

Corbel, M. 1988. Brucellosis. In Fertility and Infertility in Veterinary Practice. 4 edition. Edited by: Laing J. Bailliere Tindall: ELB. 190–221.

Corbel, M. 2006. Brucellosis in humans and animals. Geneva, Switzerland: WHO press - World Health Organization (WHO/CDS/EPR/2006.7; produced by the World Health Organization in collaboration with the food and agriculture Organisation of the United Nations and World Organization for animal health.

Cordes, D.O., Carter, M.E. 1979. Persistency of *Brucella abortus* infection in six herds of Cattle under brucellosis eradication. New Zealand Vet. J. 27:255–259.

Crawford, R.P. 1990. Epidemiology and Surveillance. In Animal Brucellosis. Edited by: Nielsen, K.H., Duncan, J.R. Boca Raton: CRC Press; 131–151.

del Pozo, J. S. G., Solera, J. 2012. Systematic review and meta-analysis of randomized clinical trials in the treatment of human brucellosis. PloS one. 7(2): e32090.

DelVecchio, V.G., Kapatral, V., Redkar, R.J. 2002. The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. Proc. Natl. Acad. Sci. USA. 99: 443–448.

El Kholy, A.A., Gomaa, H.E., El Anany, M.G., Abd El-Rasheed, E. 2009. Diagnosis of human brucellosis in Egypt by polymerase chain reaction. East Mediterr Health J. 15(5):1068–1074.

El-Tras, W., Tayel, A.A., Eltholth, M.M., Guitian, J. 2010. *Brucella* infection in fresh water fish: Evidence for natural infection of Nile catfish, Clarias gariepinus, with *Brucella melitensis.* Jvetmic. 141(3–4):321–325.

FAO, 2014. Food and Agriculture Organisation of the United Nations. World Organization of Animal Health, World Health Organization, editor. Brucellosis in humans and animals. Geneva, Switzerland.

Franco, M.P., Mulder, M., Gilman, R.H., Smits, H.L. 2007. Human brucellosis. Lancet Infect. Dis. 7:775–786.

Hassan, A., Samaha, M. 2008. Viability of *Brucella melitensis* Biovar 3, in Milk and Some Dairy Products. Egypt. J. Med. Microbiol. 17 (2):179.

Hegazy, Y. M., Ridler, A. L., Guitian, F. J. 2009. Assessment and simulation of the implementation of brucellosis control programme in an endemic area of the Middle East. Epidemiol. Infect. 137(10):1436–1448.

Hegazy, Y.M., Moawad, A., Osman, S., Ridler, A., Guitian, J. 2011. Ruminant Brucellosis in the Kafr El Sheikh Governorate of the Nile Delta, Egypt: Prevalence of a Neglected Zoonosis. PLoS Negl. Trop. Dis. 5(1):e944.

Holt, H. R., Eltholth, M. M., Hegazy, Y. M., El-Tras, W. F., Ahmed, A. Tayel, A. A., 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

Husseini, A. S., Ramlawi, A. M. 2004. Brucellosis in the West Bank, Palestine. Saudi Med. J. 25:1640–1643.

Lindahl, E., Sattorov, N., Boqvist, S., Magnusson, U. 2015. A Study of Knowledge, Attitudes and Practices Relating to Brucellosis among Small-Scale Dairy Farmers in an Urban and Peri-Urban Area of Tajikistan. PloS one, 10(2): e0117318.

Manal, E.M.E. 2011. Public Health Importance of Brucellosis In Sharkia Governorate Ph.D. thesis,

Department of Zoonoses. Fac. Of vet. Med. Moshtohor, Benha University.

Moreno, E. 2014. Retrospective and prospective perspectives on zoonotic brucellosis. Front. Microbiol. 5:213.

Morgan, W. J. B., Mackinnon, D. J., Cullen, G. A. 1969. The Rose Bengal agglutination test in the diagnosis of brucellosis. Vet. Rec. 85(23):636–641.

Nematollahia, S., Ayubib, E., Karamic, M., Khazaeid, S., Shojaeiane, M., Zamanie, R., Mansorif, K., Gholamalieeh, B. 2017. Epidemiological characteristics of human brucellosis in Hamadan Province during 2009–2015: results from the National Notifiable Diseases Surveillance System. Int. J. Infect. Dis. 61: 56–61.

OIE, 2016. Brucellosis: *Brucella abortus, B. melitensis* and *B. suis*. Terrestrial manual. Paris, France; chapter 2.1.4.

OIE, 2015. Principles and methods for the validation of diagnostic tests for infectious diseases in wildlife. In: OIE manual of diagnostic tests and vaccines for terrestrial animals. Office International des Epizooties, Paris, France. pp. 1–7.

Omer, M., Skjerve, E., Woldehiwet, Z., Holstad, G. 2000. Risk factors for *Brucella* spp. infection in dairy cattle farms in Asmara, State of Eritrea. Prev. Vet. Med. 46(4):257-265

Plumeriastuti, H., Zamri-Saad, M. 2012. Detection of *Brucella melitensis* in seropositive goats. Online J. Vet. Res. 16(1): 1–7.

Ramadan, E.S., Gafer, J.A. 2016. Comparative conventional and molecular tools for detection and differentiation of *Brucella* field and vaccinal strains. Assiut Vet. Med. J. 62 (148):13–23.

Ramadan, E.S., Ibrahim I.G. 2014. Role of Rats in spreading of *Brucella* infection in dairy farms. J. Egypt. Vet. Med. Assoc. 74 (2): 345–360. Proceedings of the 30th Arab Vet. Med. Congress, Cairo, Egypt.

Refai, M. 2002. Incidence and control of brucellosis in the Near East region. jvetmic, 90(1–4):81–110.

Refai, M. 2003. Application of biotechnology in the diagnosis and control of brucellosis in the Near East Region. World J. Microbiol. Biotechnol. 19: 443–449.

Saleh, W.M., Ghobashy, H.M., El-Bayoumi, E.M., Shalaby, M.N. 2003. Diagnosis of human brucellosis among animal farm workers and patients with fever of unknown origin using both conventional and recent techniques. 7th scientific congress 7 – 9 December, Assiut University, Egypt.

Samaha, H., Al-Rowaily, M., Khoudair, R.M., Ashour, H.M. 2008. Multicenter study of brucellosis in Egypt. Emerg. Infect. Dis. 14(12):1916–1918.

Smailnejad, G. S., Hasanjani, R. M., Janmohammadi, N., Mehraeen, R., Soleimani, A. M., Khalilian, E. 2012. Outcomes of treatment in 50 cases with spinal brucellosis in Babol, Northern Iran. J. Infect. Dev. Ctries. 6(9):654–659.

Sofian, M., Aghakhani, A., Velayati, A.A., Banifazl, M., Eslamifar, A., Ramezani, A. 2008. Risk factors for human brucellosis in Iran: a case-control study. Int. J. Infect. Dis. 12(2):157–161.

Soliman , A.S. 1998. Studies on brucellosis in farm animals with reference to public health importance in Suez Canal District. Ph.D. thesis, Faculty of Vet. Med., Suez Canal University.

Swai, E., Schoonman, L. 2009. Human brucellosis: Seroprevalence and risk factors related in Tanzania. Zoonoses Public Health. 56(4):183–187.

Wallach, J.C., Mignel, S.E., Baldi, P.C., Guernera, F.A., Goldbaum, F.A., Fossati, C.A. 1994. Urban outbreak of a *Brucella melitensis* infection in an Argentine family: clinical and diagnostic aspects. FEMS Immunol. Med. Microbiol. 8: 49–56.

WHO, 1989. World Health organization. Health Principles of Housing. Geneva, Switzerland.

Yoo, S.J., Choi, Y.S., Lim, H.S., Lee, K., Park, M.Y., Chu, C., Kang, Y.A. 2009. Seroprevalence and risk factors of brucellosis among slaughterhouse workers in Korea. J. Prev. Med. Public Health. 42:237–242.

Young, E. 1995. An overview of human brucellosis. Clin. Infect. Dis. 21(2):283–289 quiz 290.

Zayed, A.F., Abou Gazia, K.A., Gerges, M.T 2017. Detection of *Brucella* in marketable milk sold in Alexanderia city. Assiut Vet. Med. J. 63 (135): 265–296.