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Seroprevalence and Associated Risk Factors of Brucellosis in Livestock and Residents of New Valley Governorate, Egypt

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ABSTRACT

Brucellosis is a worldwide zoonotic disease which is now considered endemic in most parts of Egypt. A cross-sectional study was carried out from December 2018 to February 2020 to investigate the seroprevalence of brucellosis in humans and livestock residing in two regions located in New Valley Governorate, Egypt. A total of 1254 animals (673 cattle, 348 sheep, and 233 goats) and 523 human serum samples were examined for brucellosis using Rose Bengal test (RBT) and then randomly selected sera (15 from cattle, 7 from sheep, 3 from goats, and 45 from humans) were further analyzed by complement fixation test, enzyme-linked immunosorbent assay to compare and detect the sensitivity and specificity of RBT. The prevalence of brucellosis was 0% in cattle, sheep, and goats while it was 23.9% in humans using RBT. Concerning humans, there was a higher percentage of infection in EL Kharga (33.6%). The prevalence of this infection was also at a higher level among individuals aged above 40 years (28.57%). Furthermore, men (26.11%) were more inclined to be inflicted, compared to women (22.5%) with no significant difference. Considering the human occupation, abattoir workers were the most predominant group of people at risk (33.3%), followed by farmers (31.25%) and animal keepers (20.6%) while the lowest prevalence was demonstrated in the housewives where the prevalence was 18.8 %. As a result, risk factors of the age range, locality, time of infection, contact with animals, and occupational groups could significantly affect the prevalence of human brucellosis in the New Valley Governorate. In conclusion, brucellosis is an alarming problem among residents of the New Valley Governorate. Thus, reducing the prevalence in humans and animals in the region of study may include restriction of the marketing the raw milk and enhancing public health awareness.

Keywords: Brucellosis, Cattle, Complement fixation test, ELISA, Human, Rose Bengal test, Sheep and goats.

INTRODUCTION

Brucellosis is one of the most common worldwide zoonotic diseases, which requires major economic considerations. The reason is that it can intervene in the normal daily activities of the inflicted infected patients leading to a serious impact on public health. It can also have a detrimental effect on animal production by decreasing the reproductive efficiency, milk yield, as well as the increase of abortion (Corbel, 2006). The etiological agents causing brucellosis belong to the genus *Brucella*, and the classical zoonotic type is *Br. abortus*, *Br. melitensis*, *Br. suis*, and *Br. canis* (Pappas and Memish, 2007; Godfroid, 2017).

Humans can be infected with brucellosis by contacting animals (i.e., secretion, carcasses, or ingestion) or by consuming their products, mainly unpasteurized dairy products (Aparicio, 2013). Human symptoms mainly include undulant fever, malaise, insomnia, arthralgia, sweating, fatigue, weight loss, headache, and joint pain also, some cases may have neurological complications, endocarditis and testicular or bone abscess formation (Acha and Szyfres, 2003; Corbel, 2006). Human brucellosis proved to be a serious occupational health hazard to livestock handlers particularly abattoir workers, butchers, and veterinarians in Egypt (Zakaria et al., 2018)

Brucellosis was first reported in Egypt in 1939, but now it is endemic (Refai, 2002; Eltholth et al., 2015). In 2007, the prevalence rates of brucellosis in livestock were significantly higher in Beni Suef than other regions of Egypt (Samaha, 2008). The disease has been predominantly detected in ruminants with varied prevalence and some regions of Egypt, such as New Valley province, reported no inflicted case of Brucellosis (Wareth et al., 2014; Eltholth et al., 2017).

Isolation of *Brucella* is considered as a gold standard and the most reliable method of diagnosis; however, it is difficult and time-consuming to perform with a great risk of infection for laboratory workers, which necessitates specific biosafety measures (Mathew et al., 2015). In this regard, a variety of serological tests, such as Rose Bengal Test (RBT), Complement Fixation Test (CFT), Enzyme-Linked Immunosorbent Assay (ELISA), tube agglutination test, and buffered acidified plate antigen test can be used for the recognition of *Brucella* specific antibodies (Fatima et al., 2016). The RBT

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is simple, good, rapid, and easy to perform and can be used as a herd screening test at remote places (Teng et al., 2017; Diab et al., 2018). Moreover, CFT and ELISA can be utilized as a confirmatory test for *Brucella* diagnosis (Ashraf et al., 2014). The combination of RBT and CFT can be suggested as the best method for the diagnosis of brucellosis (Chisi et al., 2017).

The current study aimed to provide the first report of the prevalence rate of brucellosis in cattle, sheep, goats, and humans residing in New Valley Governorate, Egypt, using RBT confirmed by CFT and ELISA**.**

MATERIALS AND METHODS

Study area and period

The study was carried out in New Valley Governorate from December 2018 to February 2020. The study population consisted of cattle, sheep, goats, and humans from two regions located in the New Valley Governorate to study the seroprevalence of brucellosis.

Samples

Animal samples

A total of 1254 serum samples of farm animals (i.e., 673 cattle, 348 sheep, and 233 goats) were collected from December 2018 to December 2019, and the full history of each animal, including sex, age, season, and locality, was recorded.

Human samples

A total of 523 human serum samples were collected from patients (males or females) with the age range of 10-70 years from New Valley Fever hospital and various clinic laboratories in New Valley Governorate from December 2018 to February 2020. Demographic information of participants (i.e., gender, age, locality, and time of infliction) was also documented in the current study.

Sample collection and processing

The samples in the present study included 5-7 ml of blood from the jugular vein of the investigated animals and the cephalic vein of human cases using sterile disposable syringes. Immediately after collection of the blood sample in a sterile glass tube, the blood was left to stand still for about 30 minutes and then centrifuged at 3000 rpm for 10 minutes to obtain non-haemolyzed clear serum using sterile Pasteur pipettes followed by keeping the samples in Eppendorf tubes and labeling the tubes. The separated serum was stored in a labeled tube at -20 °C until serological examination. The number of serum samples examined from cattle, sheep, goats, and humans in two areas of New Valley Governorate are shown in table 1.

Table 1. Number of serum samples examined from cattle, sheep, goats, and humans in two areas of New Valley Governorate, Egypt

Samples	El Kharga	EL Dakhla	Total
Cattle	484	189	673
Sheep	252	96	348
Goat	186	47	233
Total	922	332	1254
Human	327	196	523

Serological test

Rose Bengal Test

All tested serum samples (i.e., 673 cattle, 348 sheep, 233 goats, and 523 humans) were examined using antigen stained with Rose Bengal and buffered to a low pH (3.65 + 0.05), the reagent was obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt. The test was performed simply by adding 25 µl of both tested serum and the reagent were placed next to the plate and then mixed thoroughly using glass rode or toothpick, shaking the plate with an electric rocker for four minutes. Then the degree of agglutination was recorded. The results were considered positive if agglutination was detected and negative if no agglutination was found.

Complement fixation test

Randomly selected samples were retested for anti-*Brucella* antibodies with CFT. Components were obtained from VSVRI, Abbassia, Cairo, Egypt, and the CFT was performed at *Brucella* Unit in Central Laboratory Evaluation for Veterinary Biologics, Abbasia, Cairo, Egypt. The test was performed according to Alton et al. (1988).

Enzyme linked immunosorbant assay

The selected samples were randomly retested for anti-*Brucella* antibodies using ELISA. The cELISA was performed by using the ID Screen® Brucellosis Serum Indirect Multi-species (ID-Vet, France) and the Human *Brucella*

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IgM ELISA Test Kit (Diagnostic Automation /Cortez Diagnostics, USA.) for animal and human sera. The results were interpreted according to the instructions of the manufactures.

Statistical analysis

Data analysis was run using the Chi-square test. The P-value is the probability of the event occurring by chance if the null hypothesis is true. P-value less than 0.05 was considered statistically significant.

Sensitivity = True Positives (TP) / [True Positive (TP) + False Negative (FN)] ×100.

Specificity = True Negatives (TN) / [True Negative (TN) + False Positives (FP)] ×100.

Ethical approval

All procedures in the current study, including human and animal sera collection, were in accordance with the Egyptian ethical standards of the national research committee. All human subjects gave their consent for the collection of the serum samples, with the agreement that any identifying details of the individuals should not be published.

RESULTS

The obtained results of RBT revealed no seroprevalence of brucellosis in cattle, sheep, and goats (Table 2). However, the seroprevalence of brucellosis by CFT and ELISA was reported 20%, and 0% in cattle, respectively. No seroprevalence of brucellosis was observed in sheep and goats using both CFT and ELISA (Table 3).

As can be seen in table 4, the analysis of seroprevalence of brucellosis in humans using RBT was 23.9%, while this rate was reported as 28.9% utilizing CFT and 31.1% employing ELISA (Table 5). As observed in table 6, the investigation of risk factors of age indicated that there was a significant relationship between human age and infection with *Brucella* ($p < 0.05$). Accordingly, the high percentage of infection was found in the age group > 40 years (28.57%) followed by the age group <40 years (i.e., 17.20 %). Regarding locality, there was a highly significant relationship between locality and infection with brucellosis in humans ($p < 0.05$), the highest infection was recorded in EL Kharga (33.6%) and the lowest was for EL Dakhla (7.7%). Although gender showed no significant relationship with brucellosis in humans, the occurrence of brucellosis was higher in males (26.11%) than females (22.5%). The finding addressing the time of infliction demonstrated that the highest percentage of infection was for days with hot weather (33.63%).

Concerning occupational factors, there was a significant relationship between contact with animals and infection**,** seroprevalence of brucellosis was higher in individuals at close contact with animals (28.4%) than those non-contacts (18.8%). Although there was no significance between the consumption of raw dairy products and infection**,** the raw milk consumers group (25.5%) showed a higher prevalence of infection, compared to non-milk consumer groups (22.4%). The highest seroprevalence was recorded in the abattoir workers (33.3%) followed by farmers (31.25%), and then Animal Keepers (20.6%), while the lowest prevalence was demonstrated in the housewives where the prevalence was 18.8 %. As tabulated in table 7, the sensitivity of RBT and ELISA concerning results of CFT in cattle, sheep, and goats was reported 0%, while the specificity of both tests was 100%. The sensitivity and specificity rates of RBT were respectively 76.92% and 100% for human participants, and the sensitivity and specificity rates of ELISA were estimated at 100% and 96.88%, respectively.

Table 2. Seroprevalence of brucellosis in animals living in New Valley Governorate, Egypt from December 2018 to December 2019 using RBT.

RBT / Farm animals	No. of examined samples	No. of Positive samples	Percentage
Cattle	673	0.0	0.0
Sheep	348	0.0	0.0
Goat	233	0.0	0.0
Total	1254	0.0	0.0

Table 3. Seroprevalence of brucellosis in animals living in New Valley Governorate from December 2018 to December 2019 using CFT and ELISA**.**

Farm animals	No. of examined samples	CFT		ELISA	
		+ve	%	+ve	%
Cattle	15	3	20	0.0	0.0
Sheep	7	0.0	0.0	0.0	0.0
Goat	3	0.0	0.0	0.0	0.0
Total	25	3	12	0.0	0.0

CFT: Complement Fixation Test; ELISA: Enzyme-Linked Immunosorbent Assay

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Table 4. Seroprevalence of brucellosis in humans residing in New Valley Governorate, Egypt from December 2018 to February 2020 using RBT.

RBT	Total (number)	+Ve (number)	Percentage
Humans	523	125	23.9%

RBT: Rose Bengal test

Table 5. Seroprevalence of brucellosis by in humans residing in New Valley Governorate, Egypt from December 2018 to February 2020 using CFT and ELISA.

	No. of examined samples	CFT		ELISA	
		+ve (Number)	Percentage	+ve (number)	Percentage
Human	45	13	28.9	14	31.1

CFT: Complement Fixation Test; ELISA: Enzyme-Linked Immunosorbent Assay

Table 6. Prevalence of brucellosis using RBT in humans regarding different risk factors in New Valley Governorate, Egypt

Species		Human (523)			Chi-square	P-value
Risk factors		Total no.	Positive no.	Percentage		
Age	<40 Y	308	88	28.57	8.987* significant at p < 0.05	0.002719
	>40 Y	215	37	17.20		
Locality	El Kharga	327	110	33.6	45.498* significant at p < 0.05	0.00001
	El Dakhla	196	15	7.7		
Gender	Male	203	53	26.11	0.8892 * Non-significant at p > 0.05	0.3457
	Female	320	72	22.5		
Weather	Hot weather	327	109	33.63	42.6856 * significant at p < 0.05	0.00001
	Cold weather	196	16	8.16		
Contact with animals	Yes	278	79	28.4	6.6563* significant at p < 0.05	0.009
	No	245	46	18.8		
consumption of Raw dairy products	Yes	255	65	25.5	0.6914 NS Non-significant at P >0.05	0.4
	No	268	60	22.4		
Occupation	Farmers	160	50	31.25	9.8809* significant at p < 0.05	0.019
	Abattoir worker	30	10	33.3		
	Animal Keeper	141	29	20.6		
	Housewives	192	36	18.8		

Table 7. Comparison of the results of RBT and ELISA for the diagnosis of brucellosis in the investigated cattle, sheep, goats, and humans as well as detection of sensitivity and specificity of both tests.

Item	RBT		ELISA	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Cattle	0.0	100	0.0	100
Sheep	0.0	100	0.0	100
Goat	0.0	100	0.0	100
Human	76.92	100	100	96.88

RBT: Rose Bengal test, ELISA: Enzyme-Linked Immunosorbent Assay

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DISCUSSION

Brucellosis is one of the most common worldwide zoonotic diseases, which requires major economic considerations, especially in developing countries, including Egypt (Afifi et al., 2005). The diagnosis is mainly based on the serological tests since it is fast, easy to use, and available, compared to other culture techniques, that are not available in laboratories of endemic countries (Young et al., 2005). The combination of serological testing should be adopted to reduce the false-negative number which contributes to the persistence of the herd problem and also to reduce the false positive number to avoid over condemnation by testing and slaughtering policy (Salem et al., 2016). Therefore, in the present study, RBT was used for the determination of brucellosis in livestock (i.e., cattle, sheep, and goats) as well as humans in the southwestern region of Egypt. As indicated in table 2, the overall prevalence rate of brucellosis in cattle, sheep, and goats was 0% using RBT.

Regarding cattle, the obtained results of the current study were in line with those obtained by Cadmus et al. (2006) and Nagi (2003) who respectively reported the seroprevalence rates of 0% and 0.70% among cattle. In contrast, (Musallam et al., 2015) and (Anka et al., 2013) reported higher rates of seroprevalence for cattle (i.e., 18.1% and 21.8%, respectively). Considering sheep, the findings of the current study yield support the results of a study conducted by Samaha (2008) who found that seroprevalence among sheep was 0.00%. In the same vein, Bekele et al. (2011) and Ebid and Salib (2020) respectively reported similar estimates of 1.2% and 0.48%, which were lower than the estimated rates of 20% and 15% respectively reported in the studies conducted by Abdel-Razik et al. (2007) and Hegazy et al. (2009). Similar to the obtained results of the current study concerning goats, (Samaha, 2008) estimated seroprevalence rates as 0.00%, which was almost near to the reported rates of 1.3% and 1.9% mentioned by Tekleye et al. (1989) and Megersa et al. (2011), respectively. However, the calculated percentages were lower than the ones recorded by Ahmed et al. (2010), Kaoud et al. (2010), Montiel et al. (2013), and Musallam et al. (2015) in the related studies (i.e., 31 %, 18.88 %, 34.3%, 38%, respectively). Accordingly, none of the farm animals were infected with brucellosis in the New Valley Governorate, which can be due to very strict measures on animal importation from outside the governorate. Natural and geographical features of the governorate are not considered an appropriate environment for *Brucella* owing to low humidity, very high temperature, and rare showers. Furthermore, most animal keepers in the country avoid locating different types of animals together in the same place.

The RBT is an ideal screening test for human brucellosis since it is a simple, rapid, and highly sensitive test for individual diagnoses (Teng et al., 2017). In the present study, the overall prevalence of human brucellosis by RBT was 23.9% (Table 4), which was similar to the obtained results of studies conducted by Yohannes et al. (2012), El-Diasty et al. (2016), and Diab et al. (2018), in which they estimated the rates as 26.6%, 21%, and 24.3%, respectively. These estimates were higher than those (i.e., 1.25%, 13.1%, 5.6%, 9.44%, 6.3%) recorded by Elmonir et al. (2016), Salem et al. (2016), Awah-Ndukum et al. (2018), Abdelbaset et al. (2018) and Ramadan et al. (2019)**,** respectively. On contrary, the reported ratios were lower than those calculated in studies conducted by Hussien et al. (2007) and Hassanain and Ahmed, (2012), which were 32.3% and 83.3%, respectively. This dissimilarity in the prevalence of human brucellosis in the current work and others may be due to different geographic locations, age range and gender distribution, variation in occupational contact, and the type of implemented tests (Alton et al., 1988). The presented data in table 6 and Figure 1 indicated that the risk factor of age can lead to significant differences. The higher percentage of infection was observed among individuals aged above 40 (28.57%). This finding was also supported by several studies, including those performed by Abdelbaset et al. (2018), Tumwine et al. (2015), and Saddique et al. (2019). In contrast, some other researchers (Nagati and Hassan, 2016; Salem et al., 2016;Tsegay et al., 2017; Saraya, 2017) reported that the highest infection rate of *brucellosis* was observed among individuals with the age range of 20-44 years, compared to the younger or older ones.

With regard to the prevalence of human brucellosis and locality, it was found that there was a significant association between the seroprevalence of brucellosis among humans and locality in New Valley Governorate (table 6 and figure 2). This finding was in line with the obtained results of a study by Nossair and Haggag, (2016), where there was a significant association between the seroprevalence of brucellosis among humans and locality ($p < 0.0001$). Addressing the effect of gender on human brucellosis prevalence, it was observed that the seroprevalence of brucellosis was higher in males (26.11%) than females (22.5%) although gender had no significant effect on the prevalence of brucellosis (table 6 and figure 3). Similarly, (El Mabrouk, 2013) found a non-significant association between the prevalence of brucellosis and gender. Analysis of the effect of weather on brucellosis showed that weather conditions had a significant effect on brucellosis with the highest infection rate during related to the seasons with hot weather (table 6 and figure 4) which was confirmed by Lolika et al. (2017) and Ayoub et al. (2019). However, (Diab et al., 2018) noticed that the highest infection rate occurred during the winter season (43.1%)**.** As can be seen in figure 5, contact with animals have a significant effect on brucellosis with the highest infection rate in individuals at close contact with animals (28.4%) than those with no contact with animals (18.8%) supported by Diab et al. (2018). Figure 6 illustrated that the seroprevalence of brucellosis was higher in the raw milk consumers group (25.5%) than non-milk consumers groups

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(22.4%) with a non-significant effect on dairy products, which was also confirmed by George et al. (2014). According to figure 7, the highest seroprevalence was recorded in the abattoir workers followed by farmers, animal keepers, and housewives indicating a significant association between different occupations and the prevalence of human brucellosis. This result agreed with those obtained by Nossair and Haggag, (2016) and Ramadan et al. (2019), who found that abattoir workers had the highest percentage followed by farmers, householders, and milker's housewives.

The obtained results of table 7 showed that the sensitivity rates of RBT and ELISA in terms of the diagnosis of brucellosis in human were 76.92% and 100 %, respectively, while the specificity of the two tests were 100 % and 96.88%, respectively, as compared with that of the CFT as a gold standard. These results were nearly similar to those reported by Shaaban et al. (2018), who found that the specificity of RBT was 97.77%, and (Rojas and Alonso, 1998), who found that the sensitivity and the specificity rates of 78.1% and 100% for RBT as well as 100% and 100% for ELISA, respectively.

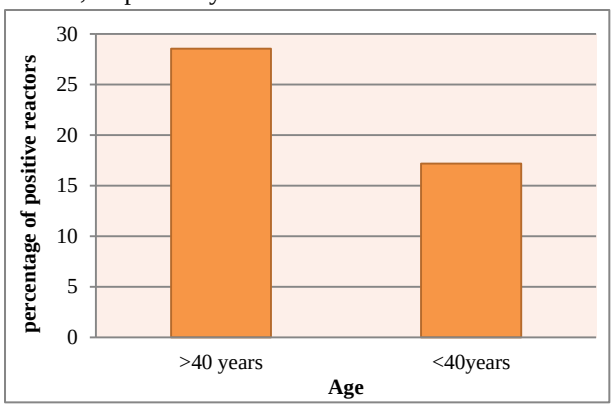

Figure 1. Seroprevalence of brucellosis in human beings using RBT regarding age groups in New Valley Governorate, Egypt

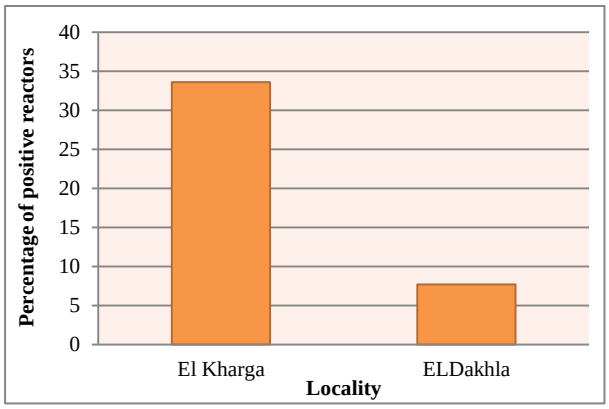

Figure 2. Seroprevalence of brucellosis in human beings using RBT regarding locality in New Valley Governorate, Egypt

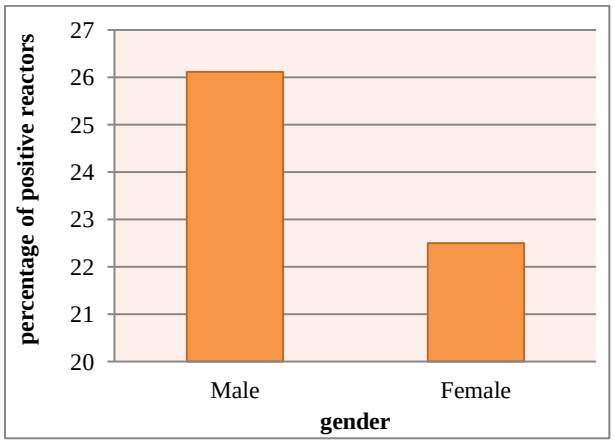

Figure 3. Seroprevalence of brucellosis in human beings using RBT in relation to gender in New Valley Governorate, Egypt

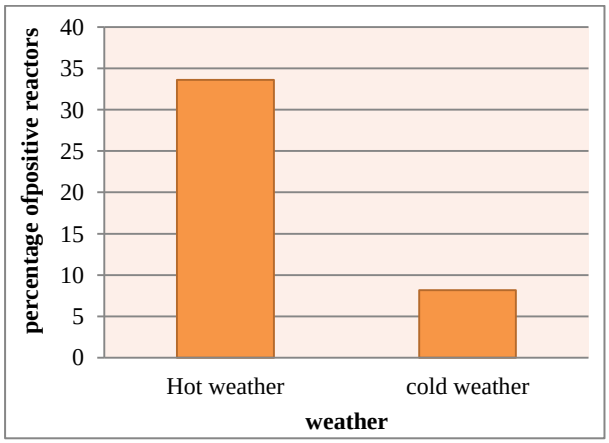

Figure 4. Seroprevalence of brucellosis in human beings using RBT regarding weather in New Valley Governorate, Egypt

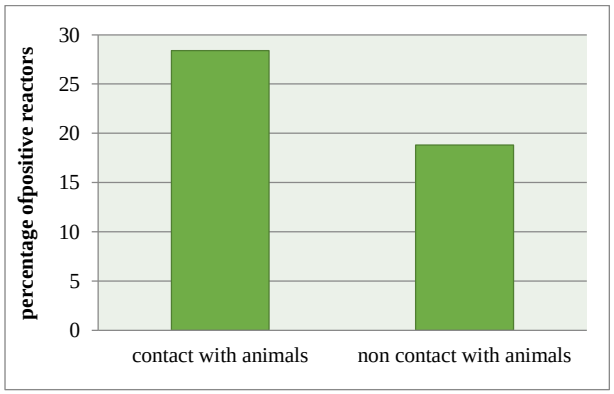

Figure 5. Prevalence of brucellosis in humans regarding contact with different animals in in New Valley Governorate, Egypt

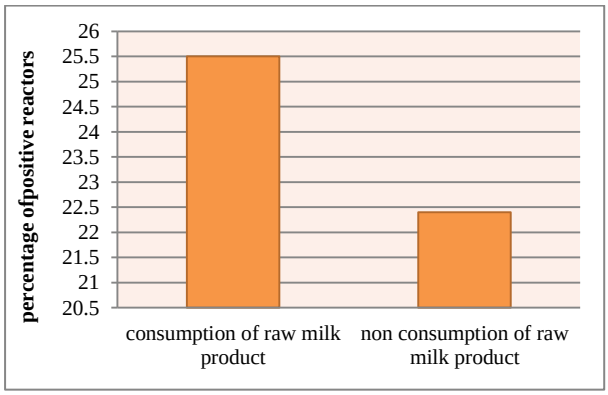

Figure 6. Prevalence of brucellosis in humans regarding consumption of raw milk product in in New Valley Governorate, Egypt

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Figure 7. Prevalence of brucellosis in humans regarding occupation in New Valley Governorate, Egypt

CONCLUSION

According to the setting and the obtained results of the current study (the first report for this area), it can be concluded that there was no reported case of brucellosis among the farm animals of New Valley Governorate. Concerning humans, brucellosis is an alarming problem in New Valley Governorate and threatens the human population through the direct and indirect transmission. There was no relationship between the recorded high prevalence of brucellosis in humans residing in New Valley Governorate and the livestock infection. The highest infection rate was recorded in Abattoir workers and Farmers than in any other occupations. Further investigation is required for other possible sources of infection. A combination of different serological tests is the best method to obtain a well-thought diagnosis of brucellosis.

DECLARATIONS

Acknowledgments

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Competing interests

All authors have no conflict of interest.

Author's contributions

Nermine A. Hassan, Ahmed M Bayoumi, Mohamed S. Diab, and Sherif Abd Allah Zidan conceived and designed the experiments. Nermine A. Hassan, Haitham Eladli and Mohamed S. Diab performed the experiments. Nermine A. Hassan, Haitham Eladli, and Sherif Abd Allah Zidan contributed reagents/ materials/ analysis tools. Nermine A. Hassan and Sherif Abd Allah Zidan wrote the paper

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