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Seroprevalence and Associated Risk Factors of Brucellosis in Sheep and Human in Four Regions in Matrouh Governorate, Egypt

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ABSTRACT

Brucellosis is a worldwide zoonosis that has major public health concern in Egypt. The present work was conducted to investigate the seroprevalence of brucellosis in sheep and human in four localities in North Western region of Egypt, on basis of the Rose Bengal plate test (RBPT) and further confirmation by complement fixation test (CFT). A total of 2471 sheep serum samples and 371 human samples were collected. The prevalence of brucellosis in sheep and human by using RBPT were 11% (272/2471) and 24.3% (90/371), respectively while by CFT were 10.56% (261/2471) and 22.91% (85/371). There was significant relationship between age and in infection rate in sheep (P<0.01), with higher percentage of infection was indicated in age group over than 24 months by 14.19% (264/1860) followed by age group less than 24 month and over 12 months by 2.39% (8/335). On studying the relation between locality and infection rate there was no significance in human samples while in sheep it was significant (P<0.01) with higher percentage of infection found in Siwa region by 20.30% (94/463) in sheep and in human by 27.6% (27/98). Concerning season there is highly significant relationship between season and percent of infection with *Brucella*, the high percent of infection found in human and sheep by 43.1% (62/144) and 16.51% (123/745) respectively and lower percent found in spring months by 8% in sheep. From our result, it is concluded that RBPT and CFT used as screening tests for detection the prevalence of species in serum samples, *Brucella* infection is found with high percent in north, west region of Egypt, which need further examination and studying another risk factor associated with infection and isolation of *Brucella* in this area.

Key words: Brucellosis, Complement fixation test, Human brucellosis, Rose Bengal plate test, Sheep

INTRODUCTION

Brucellosis is a highly contagious zoonosis caused by genus *Brucella* affecting both humans and animals (Schelling et al., 2003). Sheep brucellosis divided into typical zoonotic brucellosis that caused by *Brucella melitensis* and non-zoonotic ram epididymitis that caused by agent *B. ovis* (Acha and Szyfres 2003). Sheep and goats are primary hosts for *Brucella melitensis* (*B. melitensis*) which is common *Brucella* species in humans (Godfroid et al., 2011). Direct contact with fluids from infected animals in birthing products and other bodily fluids such as urine is the major rout of infections between animals and humans. Symptoms of *B. melitensis* in animals include abortions, stillbirths, infertility and decreased production (Corbel, 2006). While Major symptoms of human brucellosis are undulant fever, headache, muscle pain, lumbar pain and arthritis (Acha and Szyfres 2003; Pal et al., 2017).

Despite of continuous effort for zoonotic brucellosis control, that represent a major public health threats, it remains endemic in the vast majority of middle eastern countries, accused of tens of thousands of new cases yearly (Pappas and Memish 2007; Patel et al., 2017). There are about half a million new human cases of brucellosis occur every year worldwide making it the most common zoonosis (Seleem et al., 2010). Transmission of brucellosis to human occurs through ingestion of the infected product, direct contact with infected animals and its materials and through inhalation of the infected particles (Dieckhaus and Kyebambe 2017). The causative agent has a very low infectious dose, only 10 organisms of *Brucella melitensis* initiate infection (Lopes et al., 2010). Rose Bengal plate test (RBPT) is simple, good, rapid and easy to implement and can be used as herd screening test at remote places (Gul and Khan 2007; Teng et al., 2017). Moreover, Complement Fixation Test (CFT) used as a confirmatory test for diagnosis of brucellosis (Ashraf et al., 2014).

Therefore, this study was intended to study the seroprevalence of brucellosis in sheep and humans in four localities in Matrouh Governorate, Egypt by using Rose Bengal Plate Test and confirmed by complement fixation test.

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MATERIALS AND METHODS

Study area

This study completed in four regions (Matrouh, Elhamam, El dabaa, Siwa) in Matrouh Governorate, Egypt, to study seroprevalence of brucellosis, with history of non-vaccination.

Animals

This study was performed during the period between April 2016 to February 2017, a total of 2471 serum samples from Barki and Rahmani free grazing sheep in addition to 271 serum samples from humans at fever hospital in the same area of sheep rearing, the distribution of sheep and human illustrated in table 1 the data of sex and age and previous illness were recorded and there is no previous vaccination against brucellosis.

Sampling

Serum samples. In human blood samples were collected aseptically by vein puncture from each patient at the initiation of therapy and serum separated in two sterile Eppendorf that frozen for serological examination. In sheep, blood was collected (5 ml) from each sheep using plain vacutainer tube, the blood was allowed to clot at room temperature for 1-2 h, stored horizontally overnight at 4° C, then the serum was separated from the clot by centrifugation at 2000-3000 rpm for 10-15 minutes, the serum was labeled and stored at -20◦ C till tested.

Table 1. Number of serum samples examined from Barki and Rahmani sheep and human in four areas of Matrouh governorate between April 2016 to February 2017.

Locality	Humans	sheep
Matrouh	89	526
El Hamam	90	881
El dabaa	94	601
Siwa	98	463
Total	371	2471

Serological test

Rose Bengal test on human serum samples. The RBPT is a valuable screening test for diagnosis of *Brucella* (Agasthya et al., 2007) spectrum diagnostics *Brucella* Rose Bengal reagent obtained from (MDSS GmbH Schiffgraben 41 30175 Hannover, Germany) it contains ready to use standardized, killed, stained, smooth specific antigen suspensions of *Brucella* having specific reactivity towards antibodies to *Brucella* antigens which planned for quick recognition of Brucella (Melitensis, suis, and abortus) specific agglutinins (Alton et al., 1988).

Principle of the test. The smooth, killed stained *Brucella* antigen suspensions are mixed with the patient's serum. Specific antibodies to *Brucella* antigens if present in the patient serum will react with the antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of specific antibodies to *Brucella* antigens.

Rose Bengal plate-agglutination test on sheep serum samples. Prepared from heat-killed *Brucella abortus* cells (strain 99) stained with rose Bengal stain and kept in a stable acidic suspension, the antigen is produced by veterinary serum and vaccine research institute Abbasia, Cairo, Egypt, used for rapid detection of brucellosis in animals (as cattle, buffalo sheep and goat) by rapid agglutination test. The test procedure recommended by Alton et al. (1988) was followed. Briefly, 25 µl of RBPT antigen and 25 µL of the test serum were placed alongside on the plate, and then mixed thoroughly by a tooth pick or glass rod, the plate was shaken for four minutes by electric rocker and the degree of agglutination reactions was recorded. The sample was classified positive if any agglutination was observed and negative if no agglutination was noted.

Complement fixation test. The positive serum samples by RBT were retested using CFT. *B. abortus* S99 antigen for CFT was used to detect the presence of anti-brucella antibodies in the sera. The test antigen obtained from veterinary serum and vaccine research institute Abbasia, Egypt, and the CFT was done at *Brucella* unit in central laboratory evaluation for veterinary biologics, Abbasia, Cairo, Egypt according to Alton et al. (1988). The technique is usually performed using standard 96-well microtitre plates with round (U) bottoms firstly volumes of 25 µl of diluted (1/5) inactivated test serum placed in the well of the first, second and third rows. The first row is an anti-complementary control for each serum. Volumes of 25 µl of CFT buffer added to the wells of the first row to compensate for lack of antigen. Then 25 µl of CFT buffer added to third row. Serial dilutions are made by transferring 25 µl of serum from the third row onwards, 25µl of the resulting mixture in the last row are discarded. Volumes of 25 µl of antigen, added to each well except in the first row. Finally, 25 µl of complement, are added to each well. The plates are incubated at 37°C for 30 minutes, and a volume of 25 µl of sensitized sheep Red Blood Cells is added to each well. The plates are re-incubated at 37°C for 30 minutes. The results are read after the plates have been left to stand at 4°C for 2–3 hours to allow unlysed cells to settle. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis.

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Statistical analysis

Chi square test was used in statistical studies. The P value is the probability of the event occurring by chance if the null hypothesis is true, P-values 0.0001 (<0.01)

Ethical approval

All procedures performed in this study including collection of human serum samples and animals 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

In table 2 and figure 1, the overall seroprevalence of brucellosis were 11% (272/2471) and 10.56% (261/2471) in sheep serum samples using RBPT and CFT respectively while in human samples were 24.3% (90/371) and 22.91% (85/371). In table 3 and graph 1, studying age as risk factors in infection with brucellosis theirs is highly significant between age of sheep and infection with *Brucella* P<0.01, the high percent of infection found in age group over than 24 months by 14.19% (264/1860) followed by age group less than 24 months and over 12 months by 2.39% (8/335). In table 4 and graph 2, studying locality as other risk factors, theirs is highly significance between locality and percent of infection with brucellosis in sheep P< 0.01 while in human samples there is no significance. The high percent of infection found in Siwa region by 20.30% (94/463) in sheep and in human by 27.6% (27/98).

In table 5 obtained the high percent of infection found in human females by 13.6% (21/154) and in sheep by 11.35% (259/2282). In table 6 in studying human the high percent of infection found in contact animals with sheep by 31.5% 68 (216) compared with non-contact human in which the percent of infection were 14.2% (22/155) while table 7, graph 3, studying a season as a risk factors explain that theirs highly significance between season and percent of infection with *Brucella*, the highest percent of infection found in human and sheep in winter by 43.1% (62/144) and 16.51% (123/745) respectively and lower percent found in spring months by 8% in sheep and no examination to human in spring months.

Table 2. Overall prevalence of *Brucella melitensis* by rose Bengal plate test and complement fixation test in Barki and Rahmani sheep at four areas of Matrouh governorate between April 2016 to February 2017

Test	**RBPT**			**CFT**	
Species	Total No	Positive No	%	Positive No	%
Sheep	2471	272	11	261	10.56
Human	371	90	24.3	85	22.91

RBPT**;** Rose Bengal plate test, CFT**;** Complement fixation test

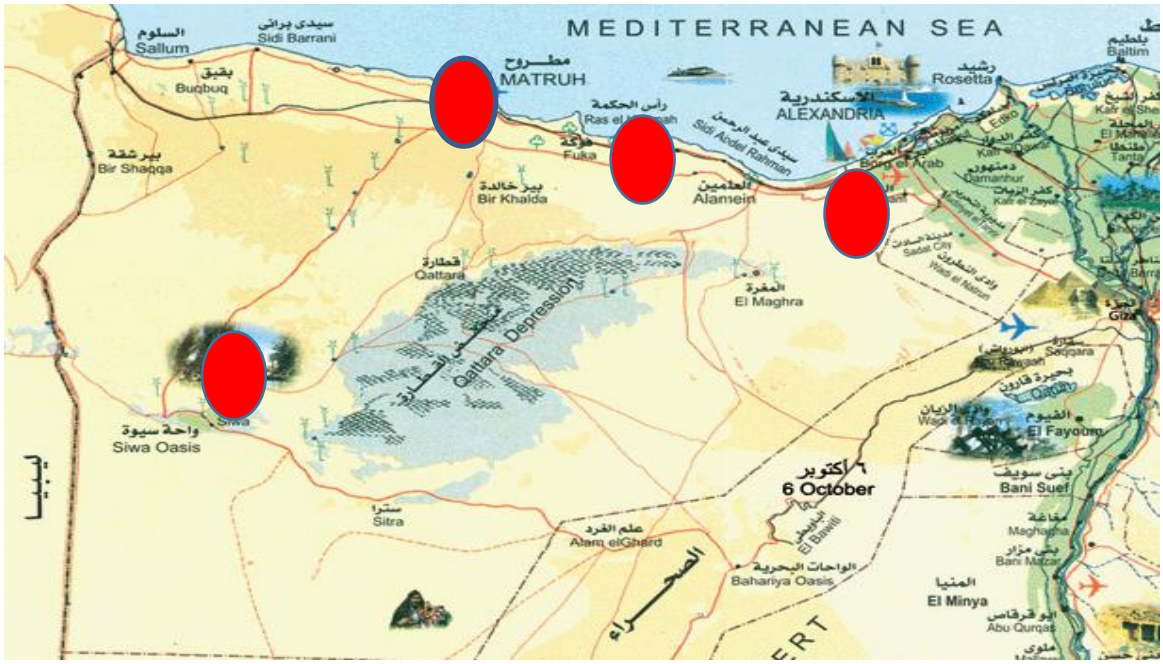

Figure 1. Map for Matrouh governorate, Egypt showing four areas of study between April 2016 to February 2017

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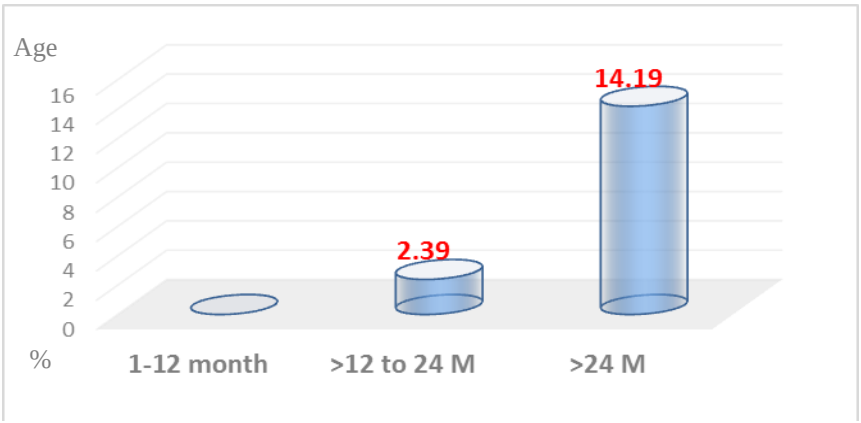
Table 3. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in Barki and Rahmani sheep at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Age (months)	Total	Positive	%
1-12	276	0	0
>12 to 24	335	8	2.39
>24	1860	264	14.19
Total	2471	272	11
Chi- square		78.82**	
P value		0.0001 (<0.01)	

** Highly significant

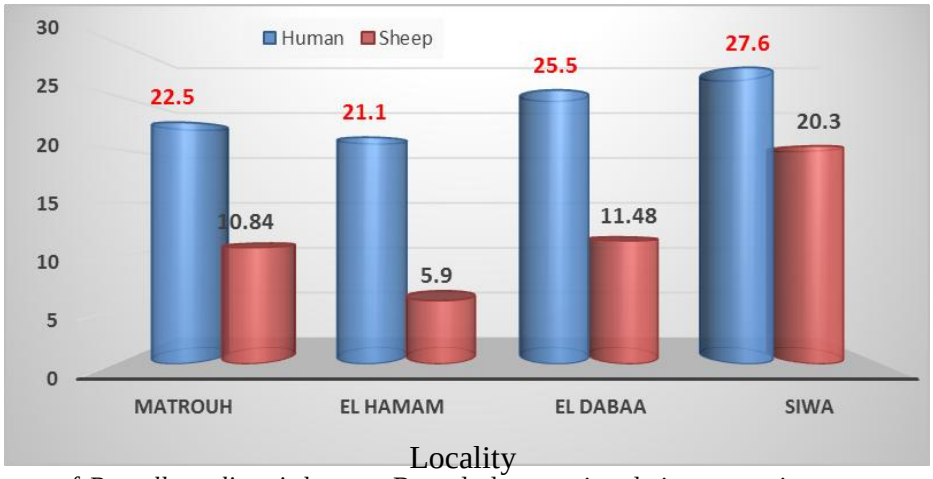


Graph 1. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in Barki and Rahmani sheep at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Table 4. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Locality \ Samples	Human serum samples			Sheep serum samples		
	Total	Positive	%	Total	Positive	%
Matrouh	89	20	22.5	526	57	10.84
El Hamam	90	19	21.1	881	52	5.90
El dabaa	94	24	25.5	601	69	11.48
Siwa	98	27	27.6	463	94	20.30
Total	371	90	24.3	2471	272	11
Chi- square		1.3NS			64.43**	
P-value		0.73			0.0001 (<0.01)	

NS: Non-Significant; **: Highly significant



Graph 2. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to age in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

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Table 5. Prevalence of *Brucella melitensis* in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017 by rose Bengal plate test in relation to sex

Samples / Sex	Human serum samples			Sheep serum samples		
	Total	Positive	%	Total	Positive	%
Male	217	69	31.8	189	13	6.88
Female	154	21	13.6	2282	259	11.35
Total	371	90	24.3	2471	272	11
Chi- square	16.17**			3.56NS		
P-value	0.0001 (<0.01)			0.06		

NS: Non-Significant; **: Highly significant

Table 6. Prevalence of *Brucella melitensis* by rose Bengal plate test in relation to history of human contact with sheep in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

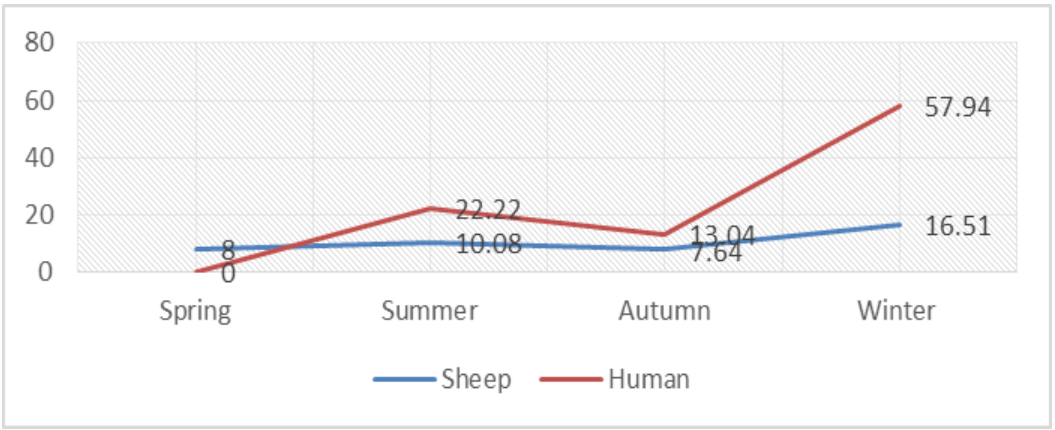
Item	Total	Positive *Brucella melitensis*	%
Contact	216	68	31.5
Non -contact	155	22	14.2
Chi- square	14.68**		
P value	0.0001 (<0.01)		

** Highly significant

Table 7. Prevalence of *Brucella melitensis* by rose Bengal plate test according to season in serum samples of Barki and Rahmani sheep and human from four areas of Matrouh governorate, Egypt between April 2016 to February 2017

Species / Season	Sheep			Human		
	Total	Positive	%	Total	Positive	%
Spring	400	32	8	-	-	-
Summer	645	65	10.08	107	16	15
Autumn	681	52	7.64	120	12	10
Winter	745	123	16.51	144	62	43.1
Total	2471	272	11	371	90	24.3
Chi- square	35.19**			46.01**		
P-value	0.0001 (<0.01)			0.0001 (<0.01)		

** Highly significant



Graph 3. Prevalence of *Brucella melitensis* by rose Bengal plate test in serum samples of Barki and Rahmani sheep and human at four areas of Matrouh governorate, Egypt between April 2016 to February 2017

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DISCUSSION

Brucellosis is a zoonosis of veterinary, major public health and economic importance in most unindustrialized countries including Egypt (Afifi et al., 2005; Asiimwe et al., 2015). The diagnosis is based mainly on the serologic testing, as it is fast and simple in addition to the fact that culture techniques are not available in laboratories in endemic countries (Young, 1995). RBPT and CFT have been used for many decades, confirming to be successful for eradicating brucellosis in some countries (Garin-Bastuji et al., 1998). Therefore, in the present work RBPT were used for determination of seroprevalence of brucellosis in both sheep and human in North West region of Egypt. Results in table 2 indicated that the overall prevalence of brucellosis by RBPT in sheep were 11% (272/2471). Our results were nearly similar to that found by Hegazy et al. (2011) who confirmed that seroprevalence among sheep were 12.2%, Hussain et al. (2014) and who concluded that the overall seroprevalence of ovine brucellosis was 10.0%. The differences in prevalence of brucellosis may be attributed to time and place of sampling in addition to people habits in reporting cases.

On the contrary, higher prevalence were recorded by Al-Majali et al. (2007) 33.1% Ahmed et al. (2010), Kaoud et al. (2010), Mahboub et al. (2013) and Nagati and Hassan (2016) by rates of 24%, 26.6%, 18.09% and 16.4% respectively. On the other hand, lower results were documented by Ferede et al. (2011), Rahman et al. (2011), Horton et al. (2014), Tsehay et al. (2014) and Patel et al. (2017) by rates of 0.74%, 3.08%, 4% , 7% and 8.70 respectively.

RBPT is the ideal screening test for human brucellosis. This is for the reason that it is highly sensitive, simple and rapid technique (Smits and Kadri 2005; Teng et al., 2017). In our study, the overall prevalence of human brucellosis by RBPT as illustrated in table 2 was 24.3% (90/ 371). Nearly similar results were obtained by Fouad et al. (1996) (26%), Kumar et al. (1997) (28.57%) and De Massis et al. (2005) who concluded that Human brucellosis is more widespread in areas where *Brucella* is prevalent in sheep. Hussain et al. (2014) confirmed that nomadic life is characterized by some factors that favor brucellosis infection such as regular migration, inadequate health services, close animal contact, poor hygienic procedures and ingestion of raw animal products.

Lower results were obtained by Afifi et al. (2007) (11%), Hassanain and Ahmed (2012) (6.26%), Hussain et al. (2014) (6%), Ali et al. (2015) (8.6%.) , Nagati and Hassan (2016) who declared that the seroprevalence of brucellosis among human was 15.2% and Tsegay et al. (2017) (4.7%). However, higher results were recorded by Ahmed et al. (2010) who founded that the overall seroprevalence of human brucellosis in Libya were 40%.

Data presented in table 3 showed that there is a highly significant difference between age groups, with higher percent of infection in age group more than 24 Month (14.19%) than other groups (2.39%) the obtained data were agreed with Abdallah et al. (2015) and Alhamada et al. (2017) who confirmed that seropositivity were significantly higher older animal ages. On the other hand, the infection rate in human were highly significant in male than female. Our results were agreed with results obtained by Afifi et al. (2005), Khan et al. (2009), Shahid et al. (2014).

A total of 2471and 371 sheep and human samples were collected from four different localities. Data presented in table 4 showed that however, there a difference in the infection rates in four localities, statistical analysis showed that significant differences found only in sheep samples. Ecological dissimilarity has been reported to influence the seroprevalence of brucellosis (Rahman et al., 2011). Prevalence of brucellosis in relation to sex were illustrated in table 5 and showed that the infection rate in sheep were higher in female (11.35%) than male (6.88%). However statistical analysis showed no significant difference. These results were agreed with that obtained by Ferede et al. (2011), Hussain et al. (2014), Tsehay et al. (2014) and Ali et al. (2015). Rams could be lower than ewes as they may be culled or sold faster. In addition to erythritol and sex hormone in ewes (Rahman et al., 2011).

On the other hand, the infection rate in human were highly significant in male than female. Our results were agreed with results obtained by Afifi et al. (2005), Khan et al. (2009), Shahid et al. (2014). Analysis of the seasonal variation of brucellosis illustrated in table 7 and showed a highly significant differences with the highest infection rate during winter season in both human and sheep. Our results disagreed with Al-Ballaa et al. (1994) who founded that the predominance of cases occurring during spring, summer and early fall and De Massis et al. (2005) who confirmed that the seasonal occurrence of human cases of brucellosis showed a peak in summer.

CONCLUSION

High prevalence of brucellosis in sheep and human in North West region of Egypt, representing major public health threats. We need further investigation including isolation and molecular identification and further analysis of other possible risk factors associated with *Brucella* infection.

DECLARATIONS

Competing interests

All authors have no conflict of interest.

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Author's contributions

Mohamed S. Diab, Yasser Elnaker, Nermin Awade, Eman Khalifa and Sherif Zidan conceived and designed the experiments. Yasser Elnaker, Nermin Awade, Eman Khalifa performed the experiments. Sherif Zidan and Yasser Elnaker analyzed the data. Mohamed S. Diab, Sherif Zidan, Nermin Awade and Eman Khalifa contributed reagents/ materials/ analysis tools. Yasser Elnaker, Mohamed S. Diab and Nermin Awade wrote the paper.

REFERENCE

Abdallah AA, Elfadil AAM, Elsanosi EM and Shuaib YA (2015). Seroprevalence and risk factors of brucellosis in Sheep in North Kordofan State Sudan. IOSR Journal of Agriculture and Veterinary Science, 8: 31-39. DOI: 10.9790/2380-08113139Acha N and Szyfres B (2003). Zoonoses and communicable Disease common to man and animal, 1 Pan American Health Organization. Washington, DC.

Afifi S, Earhart K, Azab MA, Youssef FG, El Sakka H, Wasfy M, Mansour H, El Oun S, Rakha M and Mahoney F (2005). Hospital-based surveillance for acute febrile illness in Egypt: a focus on community-acquired bloodstream infections, The American Journal of Tropical Medicine and Hygiene, 73: 392-399.

Afifi S, Wasfy M, Azab M, Youssef F, Pimentel G, Graham T, Mansour H, Elsayed N, Earhart K and Hajjeh R (2007). Laboratory-based surveillance of patients with bacterial meningitis in Egypt (1998–2004). European Journal of Clinical Microbiology & Infectious Diseases 26: 331-340.

Agasthya A, Isloor S and Prabhudas K (2007). Brucellosis in high risk group individuals. Indian Journal of Medical Microbiology, 25: 28.

Ahmed MO, Elmeshri SE, Abuzweda AR, Blauo M, Abouzeed YM, Ibrahim A, Salem H, Alzwam F, Abid S, Elfahem A and Elrais A (2010). Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006-January 2008. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 15.

Al-Ballaa SR, Al-Balla SR, Al-Aska A, Kambal A and Al-Hedaithy MA (1994). Seasonal variation of culture positive brucellosis at a major teaching hospital. Ann Saudi Med, 14: 12-15. DOI: 10.5144/0256-4947.1994.12

Al-Majali AM, Majok AA, Amarin NM and Al-Rawashdeh OF (2007). Prevalence of, and risk factors for, brucellosis in Awassi sheep in Southern Jordan. Small Ruminant Research, 73: 300-303. DOI: [10.4142/jvs.2009.10.1.61]

Alhamada AG, Habib I, Barnes A and Robertson I (2017). Risk Factors Associated with Brucella Seropositivity in Sheep and Goats in Duhok Province, Iraq. Veterinary sciences, 4: 65. DOI: 10.3390/vetsci4040065.

Ali S, Akhter S, Neubauer H, Melzer F, Khan I, Ali Q and Irfan M (2015). Serological, cultural, and molecular evidence of Brucella infection in small ruminants in Pakistan. The Journal of Infection in Developing Countries, 9: 470-475. DOI: 10.3855/jidc.5110

Alton G, Jones L, Angus R and Verger J (1988). Techniques for the Brucellosis laboratory: Paris: Institute National de la Recherdie Agrononique.

Ashraf A, El Hofy FI, Ramadan KM and Harb FE (2014). Comparative evaluation of standard serological tests for diagnosis of ovine brucellosis.

Asiimwe BB, Kansiime C and Rwego IB (2015). Risk factors for human brucellosis in agro-pastoralist communities of south western Uganda: a case–control study. BMC Research Notes 8. DOI: 10.1186/s13104-015-1361-z.

Corbel, M. J. (2006). Brucellosis in humans and animals. Geneva, Switzerland: World Health Organization, Food and Agriculture Organization of the United Nations, World Organization for Animal Health.

De Massis F, Di Girolamo A, Petrini A, Pizzigallo E and Giovannini A (2005). Correlation between animal and human brucellosis in Italy during the period 1997–2002. Clinical microbiology and infection, 11: 632-636. DOI:doi.org/10.1111/j.1469-0691

Dieckhaus KD and Kyebambe PS (2017). Human Brucellosis in Rural Uganda: Clinical Manifestations, Diagnosis, and Comorbidities at Kabale Regional Referral Hospital, Kabale, Uganda. In: Open forum infectious diseases. Oxford University Press US. pp. ofx237. doi: 10.1093/ofid/*ofx237*

Ferede Y, Mengesha D and Mekonen G (2011). Study on the seroprevalence of small ruminant brucellosis in and around Bahir Dar, North West Ethiopia. Ethiopian Veterinary Journal, 15. DOI:doi.org/10.4314/evj.*v15i2*.67692.

Fouad K, Nour El-Din A, Salah A and Murad A (1996). Study of Brucella infection as an occupational risk among abattoir workers in Alexandria. Bull Alex Fac Med, 32: 299-306.

Garin-Bastuji B, Blasco J-M, Grayon M and Verger J-M (1998). Brucella melitensis infection in sheep: present and future. Veterinary Research, 29: 255-274.

Glynn MK and Lynn TV (2008). Zoonosis update. AVMA, 233: 900-908.

Godfroid, J., Scholz, H., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., & Moriyon, I. (2011). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Preventive Veterinary Medicine, 102(2), 118–131. DOI:https://doi.org/10.1016/j.prevetmed.2011.04.007

Gul S and Khan A (2007). Epidemiology and epizootology of brucellosis: A review. Pakistan Veterinary Journal, 27: 145.

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

Hegazy YM, 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. DOI: 10.1371/journal.pntd.0000944

Horton KC, Wasfy M, Samaha H, Abdel-Rahman B, Safwat S, Abdel Fadeel M, Mohareb E and Dueger E (2014). Serosurvey for zoonotic viral and bacterial pathogens among slaughtered livestock in Egypt. Vector-Borne and Zoonotic Diseases, 14: 633-639. DOI:: 10.1089/vbz.2013.1525

Hussain M, Rind R, Adil M, Khan M, Farmanullah SA, Waheed U and Salim M (2014). Seroprevalence of Brucellosis in sheep and humans in District Kohat, Pakistan. Advances in Animal and Veterinary Sciences, 2: 516-523.

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

Khan A, Rabbani M, Muhammad K, Maqbool A and Shabbir M (2009). Seroprevalence of brucellosis in buffalo and human in Swat valley, NWFP, Pakistan. Pakistan Journal of Zoology, 9: 111-114.

Kumar P, Singh DK and Barbuddhe SB (1997). Sero-prevalence of brucellosis among abattoir personnel of Delhi. J Commun Dis, 29: 131-137.

Lopes LB, Nicolino R and PA Haddad J (2010). Brucellosis-risk factors and prevalence: a review. The Open Veterinary Science Journal, 4. DOI: 10.2174/1874318801004010072

Mahboub HHD, Helal MA, Eldaim MAA, El-Razek EMA 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, 5: 92. DOI::10.5539/jas.v5n9p92

Nagati S and Hassan SK (2016). Diagnosis of Brucella Infection in Sheep and Goat and Evaluation of the associated Practices in Animal Contacts. American Journal of Infectious Diseases and Microbiology, 4: 95-101. DOI:: 10.12691/ajidm-4-5-1

Pal M, Gizaw F, Fekadu G, Alemayehu G and Kandi V (2017). Public Health and Economic Importance of Bovine Brucellosis: An Overview. American Journal of Epidemiology and Infectious Disease, 5: 27-34. DOI: 10.12691/ajeid-5-2-2

Pappas G and Memish Z (2007). Brucellosis in the Middle East: a persistent medical, socioeconomic and political issue. Journal of Chemotherapy, 19: 243-248. DOI: 10.1179/joc.2007.19.3.243

Patel KB, Patel S, Chauhan H, Thakor A, Pandor B, Chaudhari S, Chauhan P and Chandel B (2017). Comparative Efficacy of Serological Tests for Detection of Brucella Antibodies in Sheep and Goats. Journal of Animal Research, 7: 1083-1087. DOI: 10.5958/2277-940X.2017.00161.9

Rahman M, Faruk M, Her M, Kim J, Kang S and Jung S (2011). Prevalence of brucellosis in ruminants in Bangladesh. Veterinarni Medicina, 56: 379-385.

Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M and Zinsstag J (2003). Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. Preventive Veterinary Medicine, 61: 279-293.

Seleem MN, Boyle SM and Sriranganathan N (2010). Brucellosis: a re-emerging zoonosis. Veterinary Microbiology, 140: 392-398. DOI:: 10.1016/j.vetmic.2009.06.021

Shahid M, Basit A and Khan M (2014). Prevalence of Brucellosis among the Hospital Patients of Peshawar, Khyber Pakhtunkhwa. J Infect Mol Biol, 2: 19-21. DOI:doi.org/10.14737/jimb.2307–5465/2.2.19.21

Smits HL and Kadri SM (2005). Brucellosis in India: a deceptive infectious disease. Indian Journal of Medical Research, 122: 375.

Teng Y-H, Teng J-J, Chao S, Chao H and Waghela SD (2017). Comparison of the Rose Bengal Plate and the Complement Fixation Tests with the Tube Agglutination Test for Diagnosis of Human Brucellosis. Open Journal of Clinical Diagnostics, 7: 73.

Tsegay A, Tuli G, Kassa T and Kebede N (2017). Seroprevalence and risk factors of brucellosis in abattoir workers at Debre Zeit and Modjo export abattoir, Central Ethiopia. BMC Infectious Diseases, 17: 101. DOI: 10.4236/ojcd.2017.73008

Tsehay H, Getachew G, Morka A, Tadesse B and Eyob H (2014). Seroprevalence of brucellosis in small ruminants in pastoral areas of Oromia and Somali regional states, Ethiopia. Journal of Veterinary Medicine and Animal Health, 6: 289-294. DOI: 10.5897/JVMAH2014.0331

Young EJ (1995). An overview of human brucellosis. Clinical Infectious Diseases, 21: 283-289. DOI:doi.org/10.1093/clinids/21.2.283.

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