



Cross sectional study on the seroprevalence of brucellosis in sheep, goat and man in Diyala governorate

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Abstract

Animal and human brucellosis is still endemic in many countries of the Middle East including Iraq, in spite of high efforts conducted to control the disease in animals through vaccination campaigns. Serology is the most useful and widely used tool for the diagnosis of Brucellosis in man and animals. The study was conducted from September/ 2016 to June / 2017. A total of 163 serum samples were collected from

107 and 56 adult sheep and goats of different sexes, respectively. Animals were from flocks with a history of recent or previous reproductive problems. Moreover, 84 serum samples were collected from human patients referring to different health institutes in Baqubah city with clinical signs suggestive for brucellosis (23 men and 61 women "27 none aborted and 34 aborted"). All animal serum samples were subjected to the Rose Bengal plate test (RBT) and Competitive enzyme-linked immunosorbent assay (c-ELISA), while, RBT, Rapid slide agglutination test (RSAT) and Indirect (i) ELISA were applied for human serum samples. Positive samples were detected in different percentages in various areas of the study; 97 (59.5%) out of 163 animal samples were positive in c-ELISA. However, all goat samples were negative in RBT, while 7 (9.72%), 11 (47.82%) and 1 (8.33%) of none aborted, aborted ewes and rams, respectively were positive. Aborted ewes gave a statistically significant ($p < 0.05$) higher positive reaction than aborted ones using both tests, while none aborted does give higher percentage positivity than the aborted group using c-ELISA. Concerning sex; ewes showed higher seropositive reaction than rams, while sex wise significant differences were not detected in caprine. For human serum samples; 11 (13.09%), 13 (15.47%) and 9 (10.71) were positive in RBT, RSAT, and i-ELISA, respectively. Using RBT; 2 (7.40%), 5 (13.04%) and 4 (11.76%) of men, women and aborted women gave positive results, respectively. None aborted women yielded higher seropositivity than aborted ones. Using i-ELISA; women revealed significantly ($p < 0.05$) higher seropositivity than men. In conclusion, this study approved the detection of *Brucella* seropositive reactions in animals and human samples in different areas of Diyala Governorate. Variation in the ability of various serological tests to detect animal seropositive samples was also approved.

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Key words: Seroprevalence, brucellosis, animals, man, Diyala/ Iraq.

Introduction

Brucellosis is a general term used for animal and human infections that is caused by several species of the genus *Brucella*, mainly *Brucella abortus*, *B. melitensis* and *B. suis* (OIE, 2016). Brucellae are Gram-negative, weakly acid-fast, facultative intracellular coccobacilli (Mantur and Amarnath, 2008; Gwida *et al.*, 2010). *B. melitensis* is the most virulent and most common cause of human brucellosis worldwide (Blasco and Molina-Flores, 2011).

Infection with *Brucella* is still one of the most important and widespread zoonoses in the globe according to reports of FAO, WHO and the OIE organizations (Lopez *et al.*, 2010). Brucellosis is a highly infectious, re-emerging bacterial disease of man and animals (Hadush and Pal, 2013). *Brucella* infection in animals is readily transmissible to humans, by consuming undercooked meat or unpasteurized/raw dairy products, inhalation of aerosols harboring the bacteria and through skin wounds or mucous membranes. It causes acute febrile illness which may progress to a more chronic form (OIE, 2016). Human brucellosis is a severely debilitating and disabling illness (Avdikou *et al.*, 2005) and it is still endemic in the Mediterranean basin, Middle East, Western Asia, Africa, and South America (Maurine, 2005). Small ruminant's populations in these regions showed seroprevalence values that are among the highest worldwide (Musallam *et al.*, 2016). There are about half million new human cases of brucellosis reported annually worldwide, making it the most common zoonosis (Seleem *et al.*, 2010). Brucellosis is enzootic and endemic in Iraq since 1937, and it was first isolated by an Iraqi physician (Al-Zahawi, 1938; Beattle *et al.*, 1939; Saleem *et al.*, 2010).

Serological tests are fast, safe and relatively cheap diagnostic tools; Rose Bengal plate test (RBT), complement fixation test (CFT) and enzyme-linked immunosorbent assays (ELISAs) are recommended tests for large-scale eradication purposes (OIE, 2009). The c- ELISA was developed to eliminate some of the problems arising from a residual vaccinal antibody, and from cross-reacting antibodies with some gram-negative bacteria (Poester *et al.*, 2010; Mustafa *et al.*, 2012). Some cultural habits and the close contact of animals to humans favor spread of the disease; brucellosis is of particular concern in Iraq (John *et al.*, 2008). Review of literature revealed few studies concerning human and animal brucellosis in Diyala province (Qasim *et al.*, 1995; AL-Dileamy, 2010; Fadihl and Khalil, 2016). Therefore, this study was designed to detect the seroprevalence of infection with *B. melitensis* in sheep, goats, and man in some areas of Diyala province, and to evaluate the importance of this infectious zoonotic disease in the area of the study after years of regular vaccination of animals.

Materials and Methods

Area of the study

This cross-sectional study was carried in the period extended from September 2016 to June 2017. It was conducted on sheep and goats raised in areas in and around Baqubah city; these districts are: Qarra- Tabbah, Bardiya, AL- Anbakya, Door Mandali, Kan Bani Saad and Animal farm of Faculty of Agriculture. These geographical areas were chosen according to previous and recent information of reproductive problems as for late

abortion, stillbirth, and infertility in sheep and goats. Human, samples were collected from persons referring to governmental and private health centers in Baqubah city, who were showing clinical signs suggestive of brucellosis in addition to women with recent history of miscarriage.

Collection of samples

Blood samples were collected from the jugular vein of 164 adult sheep and goats of different sex. Moreover, the median vein was used to collect samples from 84 adult men and women using disposable syringes. All blood samples were put into disposable tubes with clot activator (Orsin®), then they were centrifuged using bench centrifuge (Gemmy industrial®) at 3000 rpm for 5 minutes, serum separated and kept frozen at -20°C till used for serology .

Serological tests

All tests were performed at laboratories of the College of Veterinary Medicine / University of Diyala. Animal sera were tested by RBT (Spinract, Spain) as described by (Morgan *et al.*, 1969) and Commercial multi species c-ELISA IgG (SVANOVA Biotech AB, Uppsala, Sweden) (OIE, 2016). Human sera were tested by RBT (Cromatest ®), RSAT (Liner, Spain) as described by (Lucero and. Bolpe, 1998) and commercial i-ELISA IgG (NOVA Tec., Germany) as described by (WHO, 1998). All tests were run according to the manufacturer's instructions.

Statistics analysis

All obtained results were put into possibility tables. A Statistical Set for Social Science (SPSS), version. 22.0 (SPSS Chicago Inc.) was used to determine Chi-square test and P-value.

Results

A. Serology of animal samples

Out of 163 total animal serum samples tested, positive results were detected in 19 (11.65%) and 97 (59.5%) of samples using RBT and c-ELISA, respectively (Table- 6). The c-ELISA revealed a positive reaction in 62 (57.94%) and 35(62.5%) of sheep and goats, respectively. Statistically, a significant difference was not detected in the prevalence of brucellosis in ovine and caprine that were included in this study (Table-1; Fig. 1). Out of 163 sheep serum sample, 19 (17.75%) were positive while all goats reacted negatively to the RBT. Results for different groups of sheep (Table-2, Fig.2) declared that aborted ewes gave a statistically significant ($p < 0.05$) higher positive reaction (47.82% and 91.30%), when compared to none aborted ones (9.72% and 50%) using RT and c-ELISA, respectively. Seropositivity in different groups of sheep is significantly ($P < 0.05$) higher using c-ELISA than with RBA.

Table. 1: Result of c-ELISA for sheep and goats.

Animal species	No. of sera	c- ELISA /Positive (%)
Ovine	107	62 (57.94%)A
Caprine	56	35(62.5%)A
Total	163	97 (59.50%)

Similar capital letter A between rows do not differ at (P < 0.05)

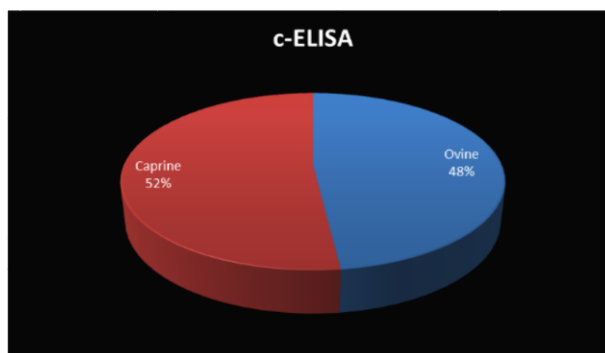


Fig.1: Seroprevalence for Brucella infection in ovine and caprine using c-ELISA.

Table. 2: Result of RBT and c-ELISA in different groups of sheep

Species of animal	No. of sera	RBT / Positive (%)	c- ELISA/ Positive (%)	X ²	P-value
None aborted ewe	72	7 (9.72%) aA	36 (50%) bA	27.88	0.001
Aborted ewe	23	11 (47.82%) aB	21 (91.30%) bB	10.26	0.01
Ram	12	1 (8.33%) aA	5 (41.66%) bA	3.55	0.05
Total	107	19 (17.75%) a	62 (57.94%) b	36.72	0.001

A&B refer to a comparison between groups at P < 0.05 (vertical) and small letters a& b refer to a comparison in groups at P < 0.05 (horizontal)

Ewes showed significantly (p<0.05) higher positivity (18.94%) than rams (8.33%) using RBT. While using c- ELISA, ewes gave none significant higher seropositivity than rams. The c-ELISA, yielded significantly (p<0.05) higher positivity than RBT in both sexes (Table-3).

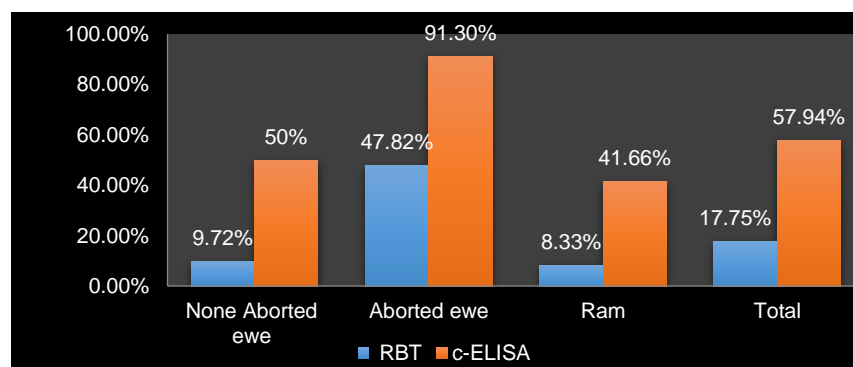


Fig.2: Result of RBT and c-ELISA for sheep.

Table. 3: Result of serological tests in sheep according to sex.

Sheep sex	No. of sera	RBT Positive (%)	c-ELISA Positive (%)	X ²	P- value
Ewes	95	18(18.94%) aB	57 (60%) bA	33.5	0.001
Rams	12	1 (8.33%) aA	5 (41.66%) bA	3.55	0.05

A&B refer to a comparison between groups at P < 0.05 (vertical) and small letters a & b refer to a comparison in groups at P < 0.05 (horizontal).

Details of c-ELISA reaction in goats are shown in (Table-4); it indicated that none aborted does give significantly (p<0.05) higher percentage positivity than the aborted group. According to sex; bucks and does gave approximately similar percentage of positivity using c-ELISA (Table-5). Total results of seroprevalence in different areas of Diyala province included in this study are shown in (Table-6, Fig. 3). A statistically significant (p<0.05) differences are detected. The highest percentage of seropositivity was detected in Bardiya and Karra- Tabba followed by Agriculture college animal farm, Door Mandli and Khan Bani Saad, the least prevalence was detected in AL-Anbakiya district.

Table. 4: Result of c-ELISA in goats.

Goats	No of sera tested	c- ELISA/ Positive (%)
None aborted does	40	26 (65%) B
Aborted does	5	2 (40%)A
Bucks	11	7(63.63%)B
Total	56	35(62.5%)

A-B (capital letters) differed significantly at P<0.05

Table-5: Result of c- ELISA in goats according to sex.

Goat sex	No of sera	c- ELISA Positive (%)
Does	45	28 (62.2%)A
Bucks	11	7(63.63%)A

Similar capital letter A between rows do not differ at (P < 0.05)

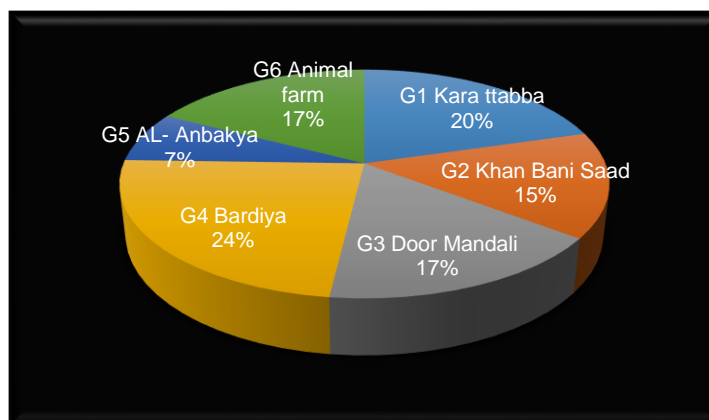


Fig.3: Seroprevalence of brucellosis in sheep and goats according to district.

Table. 6: Result of RBT and c-ELISA in animals according to district.

Animal group and district	No sera	RBT positive (%)	c- ELISA positive (%)
G (I) Kara Tabba	64	19 (29.68%)	44(68.75%)C
G (II) Khan Bani Saad	2	-	1(50%)B
G(III)Door Mandali	36	-	20(55.55%)B
G (IV) Bardiya	24	-	19(79.16%)C
G (V) AL- Anbakya	25	-	6(24%)A
G (VI) Agriculture college Animal farm	12	-	7(58.33%)B
Total	163	19 (11.65%)	97 (59.50%)

A-B& C (capital letter) differed significantly at P<0.05

B. Serology of human samples

Out of 84 human serum samples, 11(13.09), 13(15.47%) and 9(10.71%) gave positive results using RBT, RSAT, and i-ELISA (Table-7). Results for different serological tests applied for human sera are shown in (Table-7; Fig.4).

Table. 7. Result of all serological tests applied to human serum samples

Patient	NO. of sample	RBT Positive (%)	RSAT Positive (%)	i-ELISA Positive (%)	X ²	P. value
None aborted women	27	5(18.51%)aB	9 (33.33%)aB	5 (18.51%)aB	2.2	0.33
Aborted women	34	4(11.76%)aA	negative	3 (8.8%) aA	3.98	0.13
Men	23	2 (7.40%)aA	4 (13.39%) aA	1 (3.70%) aA	2.22	0.32
Total	84	11(13.09%)a	13(15.47%) a	9(10.71) a	0.83	0.65

A&B refer to a comparison between groups at P < 0.05 (vertical) and small letters a & b refer to a comparison in groups at P < 0.05 (horizontal).

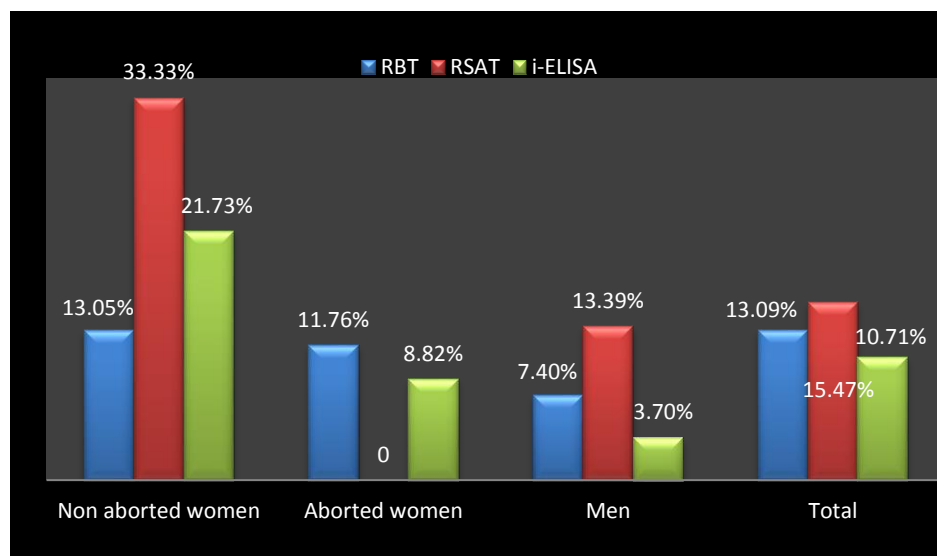


Fig.4: Percentages of seropositivity for human patients.

Using RBT; 2 (7.40%), 5 (13.04%) and 4 (11.76%) of men, women and aborted women gave positive results, respectively. By applying the RSAT, 4 (13.39%) and 9 (33.33%) were positive in men and women, respectively; while, all samples from aborted women reacted negatively to RSAT. The i-ELISA revealed positive reaction in 1 (3.70%), 5(21.37%) and 3(8.8%) of men, women and aborted women respectively. Using RBT and i- ELISA, none aborted women yielded statistically significant ($p < 0.05$) higher seropositivity than the aborted group included in this study. Significant differences were not detected between results obtained by different serological tests applied except the RSAT that failed to detect infection in aborted women.

The sex-wise result in human samples (Table-8; Fig.5) showed that using i-ELISA; Women revealed significantly ($p < 0.05$) higher seropositivity than men while using RBT and RSAT women gave a none significant higher seropositivity.

Table.8: Result of Brucella seropositivity according to sex in human

Human Patient	No. of sample	RBT Positive (%)	RSAT Positive (%)	i-ELISA Positive (%)	X ²	P. value
Women	61	9 (14.75%) aA	9 (14.75%) aA	8 (13.11%) aB	0.09	0.95
Men	23	2 (7.40%) aA	4 (13.39%) aA	1 (3.70%) aA	2.22	0.32
Total	84	11(13.09%) a	13(15.47%) a	9 (10.71%) a	0.83	0.65

A&B refer to a comparison between groups at $P < 0.05$ (vertical) and small letters a & b refer to a comparison in groups at $P < 0.05$ (horizontal).

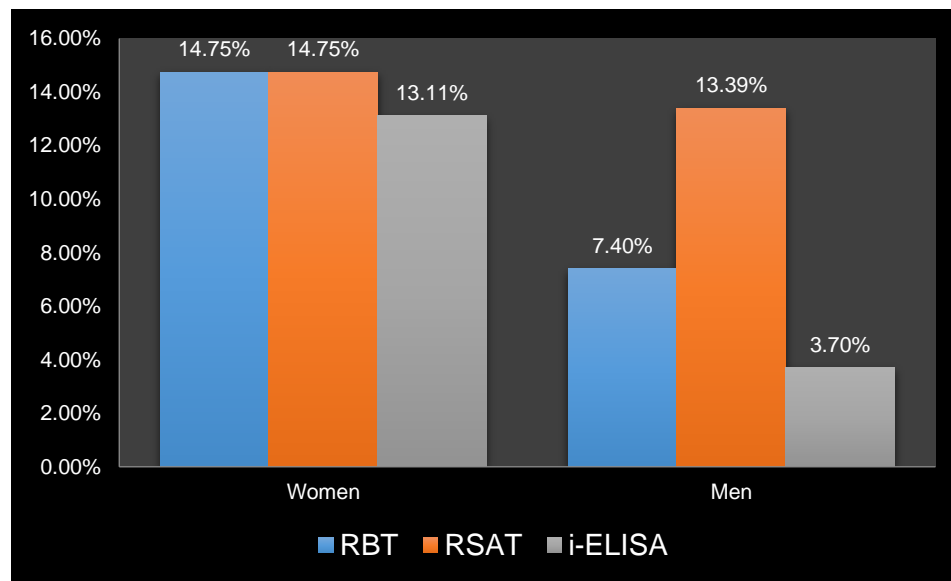


Fig.5: Seroprevalence of brucellosis according to sex in human

Discussion

It is well known that brucellosis is a highly contagious zoonotic disease affecting livestock and man. Moreover, it is worldwide in distribution and still considered as a serious problem in animals and public health for some parts of the world as

Mediterranean countries, Middle East, Arabian Gulf, Asia, Africa and Central and South Americas. In animals, brucellosis causes tremendous economic losses (Cutler *et al.*, 2005; Holt *et al.*, 2011; OIE, 2013). Conducting an accurate diagnosis for brucellosis is the cornerstone for the control of the disease in animals and consequently in man (Geresu and Kassa, 2016). Humans may be infected by any of the three classical species of the genus *Brucella*, but globally, *B. melitensis* has been and still considered the more virulent and most prevalent species that cause more severe clinical and pathological effects (Benkirane, 2006; Mantur and Amarnath, 2008; Seleem *et al.*, 2009).

In Iraq, several studies have been conducted to detect the seroprevalence of brucellosis in man and animals especially in the recent decades (Gwida *et al.*, 2010; Suadad, 2016). These studies involved nearly all Governorates in this country. However, only scarce studies found concerning this subject in Diyala province (AL-Dileamy, 2010; Fadhil and Khalil, 2016). Therefore, continuous surveys concerning the prevalence of this important zoonotic disease should be carried to establish a successful follow-up and to evaluate the control measures adopted by authorities to decrease the consequences economic losses and public health problems according to OIE recommendations (OIE, 2016).

Positive serological tests for brucellosis were found in all districts included in this study; with the highest prevalence detected in Bardiya followed by Qarra-Tabba; the least was found in goats and sheep of AL-Anbakiya district. Although there are statistically significant differences for seroprevalence with brucellosis in different areas of Diyala province included in this study, it seems that prevalence is high in all.

The seropositivity was significantly higher in c-ELISA than with RBT. This finding is in agreement with records reported previously that found ELISAs in general more accurate tests (Arslan *et al.*, 2010; Al-Abdaly *et al.*, 2012). Moreover, the RBT is considered a screening and the ELISAs are confirmatory tests, since they are more specific and sensitive (Ferreira *et al.*, 2003; Poester *et al.*, 2010; Sadhu *et al.*, 2015). Anyhow, false negative reactions can occur in the acidified antigen tests, especially in the RBT, due to the prozone phenomenon in high titer samples (Nielsen and Yu, 2010). Results of the current study showed that sheep revealed seropositivity of (17.75%) and (57.94%) using RBT and c-ELISA, respectively while in goats it was (62.5%) using c-ELISA. These percentages of seropositivity appeared to be higher than that reported previously in the same species in other parts of Iraq, such as Baghdad (AL-Azzi *et al.*, 1985; Ahmed, 2009); AL-Anbar (Al-Alousi, 2008; Al-Tae and Al-Samarrae, 2013) and Northern Iraq (Mathur *et al.*, 1974; Karim *et al.*, 1979) except Kurdistan region (Saleem, 2010).

Comparable results were reported by other workers in some Iraqi provinces as that obtained in Wasit province using RBPA (23.5%) (Al -Saaidi *et al.*, 2010), in AL-Dewania city, AL-Qadisiya province (17.39% goats, 21% sheep) by RBT (AL-Hamdani and AL-Zawadi, 2014), in Mosul city (Arslan *et al.*, 2010) ovine (8.7% RBPA, 23.6% i-ELISA), in Rutba / AL-Anbar province, sheep (17.5% RBA) (Olaiwy, 2008) in Al-alum & Biji regions in Salah EL-Din (RBA 11.95%, i-ELISA 22.82%) (Al-Abdaly *et al.*, 2013), in AL-Najaf AL-Ashraf (RBA 20%, i-ELISA 25%) (Mohamed *et al.*, 2015), in Zacho/ Duhok (i-ELISA 39.1%) (AL-Naqshebandy *et al.*, 2014). Meanwhile, a higher percentage of positivity was detected in other studies conducted in Iraq (Al-Farwachi *et al.*, 2010; Salman and Musa, 2015; Ahmed *et al.*, 2016). This wide variation in the prevalence of brucellosis in different Iraqi provinces run parallel with that obtained by

others researchers worldwide (Dean *et al.*, 2012). It is concluded that incidence of brucellosis varies widely not only between countries but also within countries; and they suggested that demographic, occupational, cultural; and socioeconomic factors may play a significant role in this variation. Variations in the percentage of seropositivity among animals were also recorded in different areas of the same province (Ljung, 2013; Salman and Mosa, 2015).

In this study, statistically significant ($p < 0.05$) difference was not found in ovine and caprine *Brucella* seropositivity. It is well known that both sheep and goats are the primary natural reservoir host for *B. melitensis* (WHO, 2006; Godfroid *et al.*, 2010). Moreover, it is supposed that goats are more susceptible to infection than sheep (Timoney *et al.*, 1988; Karim *et al.*, 1979; Al-Ani *et al.*, 2004; Ahmed *et al.*, 2010; Kaltungo *et al.*, 2013; AL-Hamdani and AL-Zawadi, 2014; Ebrahimi *et al.*, 2014). In contrast, other studies found that goats were less liable for infection with *Brucella* than sheep using serological tests (Ismaily *et al.*, 1988; Ismaiel, 2005; Gul and Khan, 2007; Ljung, 2013; Islam *et al.*, 2013; Sadhu *et al.*, 2015).

It is worth to mention that in some areas of the world the disease is more important in sheep than in goats; like most countries bordering the Mediterranean Sea and in Southwest Asia, where the fat-tailed sheep as Awassi are reared (EUROPEAN COMMISSION, 2001). Using RBT and c-ELISA, aborted ewes yielded significantly higher seropositivity than none aborted ones; this is logical since positive serological tests are associated with cases of abortion more than with the not yet aborted ones. In contrast, aborted does reacted less with the c-ELISA than none aborted ones; this may be partly explained by a recent abortion attack where more time is needed for development of antibody titer.

Sex-wise significant differences were not detected in *Brucella* seropositivity between male and female sheep and goats. Anyhow, there are controversial reports regarding the prevalence of brucellosis and its relation to sex of animals, and our finding runs parallel with that obtained by previous workers, who found no detectable connection between susceptibility to *Brucella* infection and sex of exposed animal or man (Muma *et al.*, 2006). Others believed that males of small ruminants are more affected with brucellosis than females (Al-Tae and Al-Samarrae, 2013; Ahmed *et al.*, 2016; Alrodhan, 2017). While some scientists found that females are more liable to infection with brucellosis than males (Hussein *et al.*, 2005; Al-Alousi, 2008; Olaiwy, 2008; AL-Hamdani and AL-Zawadi, 2014; Hussain *et al.*, 2014; Salman and Mosa, 2015). Positive results were detected in 11 (13.09%), 13 (15.47%) and 9 (10.71%) of human samples using RBT, RSAT, and i-ELISA, respectively and statistical differences haven't been detected between the results of the three tests. Using RBT and i-ELISA; none aborted women showed significantly ($p < 0.05$) higher seropositivity for *Brucella* than none aborted ones. This finding agreed with that recorded in Jordan (Abo-Shehada and Abu-Halaweh, 2011), who found no statistically significant difference between anti-*Brucella* antibody titer among women with miscarriage and those with no history of miscarriage using RBT and CFT. Additionally, Makhseed *et al.*, (1998) related intrauterine fetal death in women with brucellosis to acute signs of illness rather than to transplacental infection. Although *Brucella* spp. are the important cause of abortion in animals, for human this is still controversial. Besides, many reports have excluded *Brucella* from being a cause of miscarriage in women due to lack of erythritol in women's placentas and the presence of

anti-Brucella activity in human amniotic fluid (Nassaji *et al.*, 2008). In rare cases, Brucella spp. were isolated from fetal or placental tissues, but it has not been demonstrated that brucellosis causes abortions more frequently than do other bacterial infections (Poole *et al.*, 1972; Young, 1983). In a study from 1983 through 1995 on brucellosis in women with birth problems in Saudi Arabia, Khan *et al.*, (2001) suggested that Brucella species may indeed produce human abortions more frequently than do other bacterial pathogens.

The human sex-wise results in the current study detected significantly ($p < 0.05$) higher infection rate in women than men using i-ELISA. Moreover, statistically significant differences were not found between the two sexes using RBT and RSAT. This finding is in agreement with many studies conducted in other parts of Iraq (AL-Khafaji, 2003; Tofah, 2008; Al Zubaidy, 2008; Mohammed, 2009; Al-Hussain and Thaer, 2012; Rasul and Mansoor, 2012) and in other parts of the world as in India (Din *et al.*, 2013) in Yemen (Al-Haddad *et al.*, 2013; Al-Arnoot *et al.*, 2017), in Turkey (Cetinkaya *et al.*, 2005; Apan *et al.*, 2007 ; Pabuccuoglu *et al.*, 2011) in Ethiopia (Yohannes *et al.*, 2012), in Pakistan (Hussain *et al.*, 2008; Din *et al.*, 2013), in Mangolia (Tsend *et al.*, 2014), in Kenya (Nakeel *et al.*, 2016) and in Brazil (Soares *et al.*, 2015).

Other researchers found that men are more prone to Brucella infection than women such as those obtained in Baqubah city (AL-Dileamy, 2010), Libya (Ahmed *et al.*, 2010), India (Metri Basavaraj *et al.*, 2011; Singh & Parikh, 2014; Sharma *et al.*, 2016), Iran (Ruiz-Mesa *et al.*, 2005; Ebrahimpour *et al.*, 2012), Ethiopia (Tsegay *et al.*, 2017), Malaysia (Jama'ayah *et al.*, 2011; Bamaiyi *et al.*, 2017) and from China (Wu *et al.*, 2013; Lai *et al.*, 2017). While, others claimed that sex predicts no effect on susceptibility to Brucella infection in humans (Sümer *et al.*, 2003; Muma *et al.*, 2006; Alim *et al.*, 2015). Nevertheless, it has been explained that males are more susceptible to infection than females because of occupational exposure risk that comes from the rearing of the animal. While women are more concerned with caring for house and children (Bikas *et al.*, 2003; Mantur and Amarnath, 2008; Cekanac *et al.*, 2010; Metri Basavaraj *et al.*, 2011; Agasthya *et al.*, 2012; Wu *et al.*, 2013). In Libya, Ahmed *et al.*, (2010) explained higher infection rate among males in Yafran district by the fact that in the culture and tradition of that region raw milk is consumed more frequently by men.

Since pastoralism and living in rural areas is not usually associated with laboratory diagnostic abilities, in addition to limited access to veterinary and public health facilities; brucellosis is likely to remain untreated in many nomadic settings, with both humans and livestock being infected (Racloz *et al.*, 2013). Moreover, there is a high possibility of persistence of brucellosis in rural areas like that found in Diyala districts and different parts of Iraq; in spite of various efforts established by governmental veterinary and human health authorities for the control of this important zoonotic disease .

In conclusion, this study approved the detection of Brucella seropositive samples in different areas in Diyala province/ Iraq. Moreover, the variation in the ability of various serological tests to detect the animals and human seropositive samples was also approved. The study also determined the zoonotic nature of this infectious disease in the areas of the study after years of regular vaccination of animals.

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