Sero-prevalence of Brucellosis in goat and sheep's herds in Raparin District, Kurdistan region, Iraq

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ABSTRACT:

Brucellosis is a worldwide disease caused by an infectious bacterial disease called Brucella, it has some species in genera. Prevalence of the brucellosis in many area of the world particularly in some Mediterranean and Middle East countries. A total 494 sera samples were collected from 36 herds in Raparin district including 113 human sera (herder), 199 sheep and 182 goat sera sample.Using three serological such as Rosbengal test (RBT), 2-Mercaptoethanol test (2ME) and Enzyme linked immune sorbent Assay (ELISA) detect the prevalence of brucellosis in the district according to animal species, gender, vet office area and age group with a comparison between serological tests which was used in this study during august 2017 till jun 2018 . All samples collected from adults of both sexes from different seven geographical areas in Raparin district. The total prevalence of brucellosis was 26.3% this value varied according to ifferent areas. The highest in Sangasar and Bngrd was 37.3%, and 35.3% while the lowest in Rnia and Zharawa was 13.2%, and 16.9% respectively, using the Rose-bengal test as a screening test to identify the prevalence of the disease. The total prevalence was varied according to the animal species, gender and age group. The study revealed that the prevalence of the disease varied according to the used type of the serological tests. RBT recorded the highest 26.3% then the 2-Mercapto-ethanol test recorded (14.4%) and ELISA recorded (12.4%), When comparing the positive results of ELISA and other serological tests the results reveals significant ($P<0.05$) difference between ELISA and other serological tests.

Key words: Brucellosis, RBTP, 2ME, ELISA, Goats and Sheep

INTRODUCTION:

Brucellosis is a disease that is thought to have existed since ancient times, as it was first described more than 2,000 years ago by the Romans and Hippocrates. Brucellosis was firstly discovered and reported in 1863 by Jeffrey Alien Marston, who was a surgeon in the British Military. (Manish *et al.*, 2013)

Brucellae are small coccobacilli which have dimension (0.5-0.7 μm) width and and long (0.6-1.5 μm), gram-negative, arranged singly and sometime in pairs or small groups. The morphology of bacteria is stable excluding old cultures, where appear as pleomorphic. They are non-spore forming, non-motile, and true capsules, flagella and pili are not formed by bacteria. The smears of liquid or solid specimens are colored red by the modified Ziehl-Neelsen stain that is occasionally used for the microscopic diagnosis of disease (Garrido et al., 2001)

They are aerobic and carboxyphilic; and are facultative, pathogenic and intercellular organisms with a tendency for organs rich in sugar erythritol, such as the reticuloendothelial system and the reproductive tract(Carter G.R., 2004). The genus Brucella (Class Alphaproteobacteria, Order Rhizobiales, family Brucellaceae) has 6 species including: *Br. melitensis, Br. abortus, Br. canis, Br. ovis, Br. suis, and Br. neotomae* (Christopher, Umapathy and Ravikumar, 2010).

Brucellae's major distinguishing antigens include the smooth and rough lipopolysaccharide (S, R-LPS) and two associated polysaccharides: native hapten (NH) and B-polysaccharides (poly B), and at least 20 protein or glycoprotein antigens(WHO, 1986).

Several serological studies were conducted on Brucellosis in the north province of Iraq including, Erbil, Kirkuk, Sulaimani, Duhok, and Nainava(Shareef, 2006).

The prevalence of the disease in Iraq had been varied among researchers depending on the type of tests which used in the same region and in different regions, as well as depending on the geographical location, which conducted the study, size and type of the samples, (Al-Hankawe and Rhaymah, 2012) conducted to compare between the ELISA and other serological tests (RBT, mRBT, TAT and 2-mercapto-ethanol), for diagnosis of Brucellosis in sheep.

There are various serological assays available for measuring antibody following infections, applied for diagnosis of Brucellosis are: serum agglutination test (SAT), Rose Bengal plate test (RBPT), 2-mercapto-ethanol test (2-MET), milk ring test (MRT), buffered antigen test (BPAT), and complement fixation test (CFT). Others include the card test (CARD), Rivanol test, Coombs test, indirect immune-flourescent test (IFAT), heat inactivation test (HIT) and immune assay and enzyme-linked immunosorbent assay (ELISA) (Kaltungo *et al*, 2014)

Enzyme-linked immunosorbent assay (ELISA), the Serodiagnostic, colorimetric test, depends on antibody detection, that is produced in response to lipopolysaccharide (LPS) or whole bacterial extracts(Godfroid *et al.*,

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2011). Brucella detection by ELISA is more sensitive than other serological tests that are used for the detection of brucellosis(Al Dahouk, 2013).

The addition of 2-Mercaptoethanol (2-ME) destroy IgM and leave IgG for agglutination reaction. The test is not as sensitive as the standard tube agglutination test, but the results correlate better with the activity of the disease (Brooks et al., 2004). It is regarded superior to other tests in the determining of the efficacy of antimicrobial therapy(Madkour, et al., 2002).The Aim of the present study are detection seroprevalence of human and animal brucellosis by using different serological tests and finding the significant differences among of them in accuracy and efficiency.

Material and Method

Study design and sample collection:

From August 2017 to Jun 2018, this study was carried out to detect Brucellosis in small ruminants with a record of recent abortion and to isolate Brucella from seropositive aborted animals in Raparin district. Sheep and goats with a history of recent abortion case were selected based on the information obtained from the veterinarian and owners, also the herdsman's who have a direct contact with the animals.

Rose Bengal Test (RBT)

The test was described by Blasco et al. (1994) as follow:

At the first Rose Bengal antigen and Sera wormed at room temperature 22 ± 4°C. 75 µl from sample with positive and negative control used on a white plate. The antigen container should be shacked well and putting 25 µl of it beside every serum spot. When the last drop of antigen has been putted to white plate, immediately must be mixed the antigen and serum completely by a means of a clean stick for each test to produce a circular zone of 2cm in diameter. Gently agitate the mixture for 4 minutes at room temperature. After the 4 minutes has been finished, the results immediately were read. Any visible agglutination is regarded to be positive.

Enzyme Linked Immuno Sorbent Assay (iELISA)

The serum samples to be tested are diluted and incubated in the wells of microtiter plates coated with Brucella lipopolysaccharide (LPS), upon incubation the Brucella specific antibodies form immunecomplexes with Brucella LPS then washing away the unbound material.

When an anti-ruminant antibody enzyme conjugate is added, this can bind to any immuno –complex Brucella antibodies. Unbound conjugate is washed away with washing solution and the enzyme substrate (TMB) is added. In the presence of the enzyme, the substrate is oxidized and develops a blue compound that becomes yellow in

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color after blocking. The subsequent color development is directly related to the amount of antibodies to Brucella present in the test sample.

Calculation the results performed by the validity of the assay was checked. The positive control means (PC X) must be greater than or equal to 0.350 optical density (OD). As well as the ratio between the positive controls mean (PC X) and the negative control (NCA 450) must be greater or equal to 3.00 to calculate the sample to positive (S/P) percentage for each sample.

2-Mercaptoethanol test (2-ME).

The test is carried out simultaneously with and in the same manner as the standard agglutination test, each serum was treated with equal volume of 0.1ml of diluents solution (1/10) of 2-Mercaptoethanol while control dilutions were treated with sterile normal saline. Antigens (Rose Bengal test) were allowed to reach room temperature and gently shacked to disperse the particles. Three drops of serum suspension (serum – 2-ME) were placed on three separated circles of slide. One drop of antigens (Rose Bengal antigen) was added to each circle respectively. Mixed well using plastic stick, slide was shacked gently for 4 minute and observed for any agglutination within 4 minute exactly.(Buchanan and Faber, 1980).

Result and Discussion

Determining of Sample size

Raparin district composed of seven veterinarian administrative offices from (Rania, Qaladze, Zharawa, Sangasar, Bngrd, Hajiawa and khdran). Small ruminant livestock (sheep and goat) population equal to more than 330000 according to the dean veterinarian office report which that shown in table (1), in these population 1025 cases from sheep and goats with a history of recent abortion case with their herders that they had direct contact with livestock were selected as a susceptible case for present research based on the information obtained from the veterinarian office and owners.

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Table (1): Presence rate of livestock in Raparin district, with susceptible case rate according vet administrative office.

Area	Type of livestock	No. of livestock	Total	No. of livestock in study area	No. of susceptible case
Rania	Sheep	21449	58127	1405	139
	Goat	36678			
Qaladze	Sheep	36024	54044	1341	85
	Goat	18020			
Zharawa	Sheep	32252	45256	1130	179
	Goat	13004			
Bngrd	Sheep	22789	27386	1825	104
	Goat	4597			
Sangasara	Sheep	47182	83696	1110	167
	Goat	36514			
Hajiawa	Sheep	22164	37790	1570	163
	Goat	15626			
Khdran	Sheep	16270	26642	1610	188
	Goat	10372			
Total	Sheep	198130	332941	9991 (%3)	1025 (%10.3)
	Goat	134811			

The sampling was carried out purposely by selecting individual pastoral with a history of recently aborted goats and sheep and proportional number of aborted goats and sheep was estimated in order to select the required number of study animals from each vet administrative office of Raparin district.

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The sample size for serological study was estimated based on our previous questionnaire report of 10.3 % suspected case in aborted small ruminant in Raparin district (Table 1). Therefore, the sample size was calculated using the formula described by(Thrusfield, 2006), with defined precision of 5 % and level of confidence interval of 95%.

$$n = \frac{1.96^2 \, Pexp\,(1-Pexp)}{d^2}$$

n=required sample size,

Pexp=expected prevalence, and

d=desired absolute precision

Hence, based on the above formula, taking the expected prevalence of brucellosis in aborted cases as 10.3% (Table 1), a desired absolute precision of 5% and 95% confidence level, 414 animals are required.

$$n = \frac{3.84 \times 0.1(1-0.1)}{(0.05)^2} = 138$$

Therefore, about 138 samples of each part such as suspect human and aborted small ruminants (sheep and goat) were considered for this study, in total about 414 samples at least were considered from selected veterinarian administrative in Raparin district. Also according to the Slovin's formula our study must composed from about (400) samples(Glenn D, 1992).

$$n = \frac{N}{1+Ne^2}$$

N= population size = 332941 Table (4.1)

e= margin on error = 0.05

n= Number of sample

$$n = \frac{N}{1+Ne^2} = \frac{332941}{1+332941(0.05)^2} = \frac{332941}{833.352} = 399.5$$

Prevalence of Brucellosis according to animal species:

The study revealed differences in the infection rates of brucellosis between human, sheep and goats. Out of 494 sera tested, 113 (22.9%) from human sera, 199 (40.3%) from sheep and 182 (36.8) from goat sera. were tested with RBT (Table 2 and fig. 1)

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Table (2): Prevalence of Human, sheep and goat brucellosis by using RBT according to animal species

	Total	Results	% Percentages
Human	113	positive ٤٢	37.2%
		71 Negative	62.8%
Sheep	199	positive ٥٦	28.1%
		143 Negative	71.9%
Goat	182	positive ٣٢	17.6%
		Negative ١٥٠	82.4%
Total	494	positive ١٣٠	26.3%
		Negative ٣٦٤	73.7%

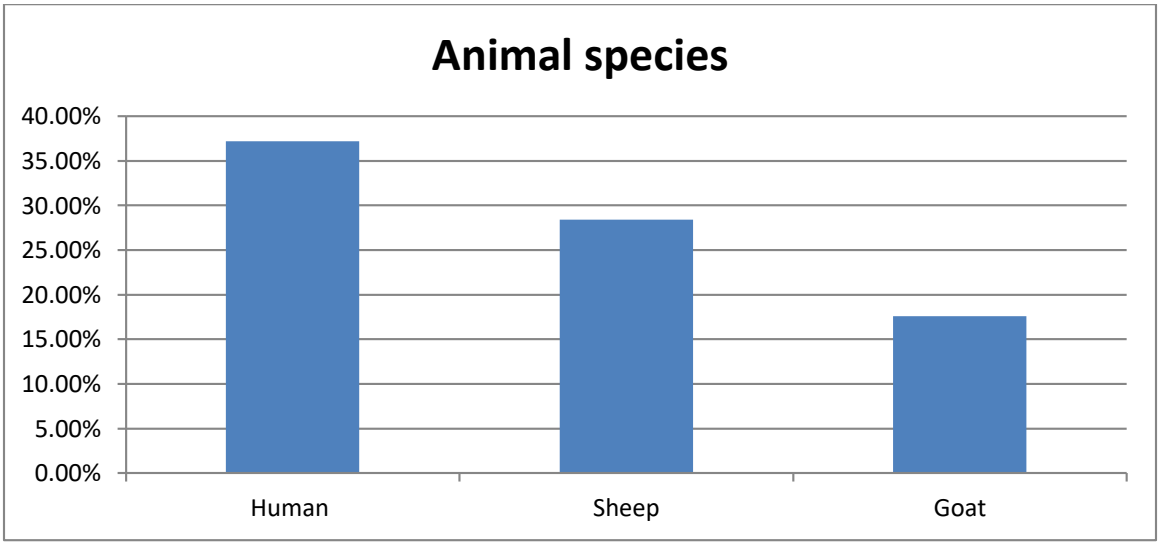

Fig. (1): Prevalence of Human, sheep and goat brucellosis by using RBT according to animal species

Current results showed that the human brucellosis had a high level rate 37.2%, sheep brucellosis were 28.1% and goat brucellosis were 17.6%, results obtained by RBT according to animal species. This results was agreed with others found: (Kaoud *et al.*, 2010)26.66%, 18.88% and 17.22%in Eygpt, (Ahmed *et al.*, 2010) 24%, 31% for sheep and goat brucellosis respectively in western mountain region in Libya. On the other hand some articles result disagree with current results due to low prevalence rate of brucellosis such as, (Hegazy *et al.*, 2011)10.4%, 9.7% in Kafr El Sheikh-Egypt; (Golo dabbasa, 2013) 1.75%, 2.8% in Yabello district, Ethiopia; (Akbarmehr and

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Ghiyamirad, 2011) 4.18%, 5% in Sarab city, Iran; (Diab *et al.*, 2018)5.71%, 10% in Eygpt;; (Bertu *et al.*, 2010) 9.3%, 10.1% in Plateau state, Nigeria; (Hawari, 2012) 21.1% in south province of West in sheep and goats respectively.

As a result potential transmission of the disease was increased in long-term close contact such as behavior of ovine animals together in parturition or at night(Garrido-Abellan, *et al*., 2001). Keeping in contact sheep with goat consider as a risk factor for brucellosis transition(Coelhoa and , AC Coelhob*, 2013).

Prevalence of brucellosis according to vet administrative office.

According to current results, the higher seroprevalence 25(37.3%) from 67samples was found in Bngrd vet Office with significant differences ($p \leq 0.05$), and lower seroprevalence 9(13.2%) from 68 samples was recorded in Rania, as shown in (Table 3 and Fig. 2):

Table (3): Prevalence of brucellosis by using RBT according to the vet office

Vet office	No of livestock	susceptible case	No. of sample	Rate of positive
Rania	1405	139	68	9(13.2)
Qaladze	1341	85	73	14(%19.2)
Zharawa	1130	179	71	12(16.9%)
Sangasar	1825	104	67	25(37.3%)
Bngrd	1110	167	68	24(35.3%)
Hajiawa	2070	163	73	23(31.5%)
Khdran	1610	188	74	23(31.1%)
Total	991	1025	494	130(26.3%)

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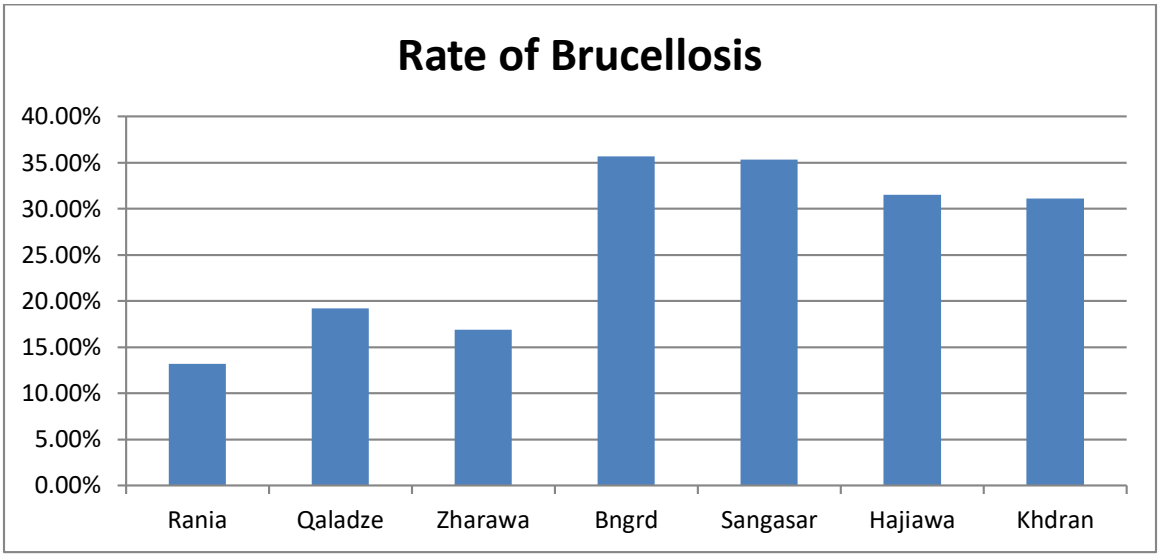

Fig. (2): Prevalence of sheep and goat brucellosis by using RBPT according to the vet administrative office.

The present results showed that the prevalence of the brucellosis variable according to the location, the ratio was between 13.2% and 37.5%, these results agreed with (Stamatiou *et al.*, 2009) that he Saied the susceptibility of animals to brucellosis depends on many factors including geographical variability, population density (number of animals to land area) which was attributed to increased contact between susceptible and infected animals(Radostits *et al.*, 2007).The existing or traditional husbandry practices of handling multiple species supports the spread of brucellosis in the area(Tesfaye *et al.*, 2012).

Farmers in Raparin district usually keep their animals for breeding and milk production purposes than for meat production, some authors have reported that the brucellosis is more prevalent in milk than in meat herds (Omer *et al.*, 2000), and (Lithg-Pereira, *et al.*,, 2004). The majority of goat and sheep flocks are movable. Transporting of infected ruminants from area to area may induces the pastures to be contaminated and resulting in the increasing the prevalence of disease to other animals, other herds and areas. This transportation of animal flock is a most important risk factor for un-success of controlling and eradicating of a disease programs (Samaha *et al.*, 2008).

A total result obtained according to RBT in present study was 26.3% positive, it was agreed with results recorded by (Shahaza *et al.*, 2009) who found 26.4% in sheep in Malaysia; and disagree with the results that obtained at low rate by (Gumaa *et al.*, 2014) who found 3.4% in sheep in Kassala state, Eastern Sudan; (Tesfaye *et al.*, 2012) who found 4.6% in sheep and goats in Adamitulu-Jido-Kombolcha District-Ethiopia;(Jackson *et al.*, 2007), who recorded 5.5% in goats in Tajikstan and (Iqbal et al., 2013)who found 7% in sheep in Southern Punjab-Pakistan; (Adugna, *et al.*, 2013)who found 8.33% in sheep and 15% in goats in Afar National Regional State, Northeast Ethiopia.

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Prevalence of Brucellosis according to gender:

Results showed none significant differences ($p > 0.05$) in infection rates of total male female study constituent which were 28.7% male rate and 25.2% female rate in totally. However, significance observed between male and female infection of human and sheep, while none to goats (Table 4 and fig.3).

Table (4): Prevalence of sheep and goat brucellosis by using mRBT according to gender.

Type of sample	Gender	susceptible case	Positive		Negative	
			No.	%	No.	%
Human	Male	62	26	41.9%	36	50.1%
	Female	51	16	31.4%	35	68.6%
Sheep	Male	60	13	21.7%	47	78.3%
	Female	139	43	30.1%	96	69.9%
Goat	Male	42	8	19.1%	34	80.9%
	Female	140	24	17.1%	116	82.9%
All animal species	Male	164	47	28.7%	117	71.3%
	Female	330	83	25.2%	247	74.8%

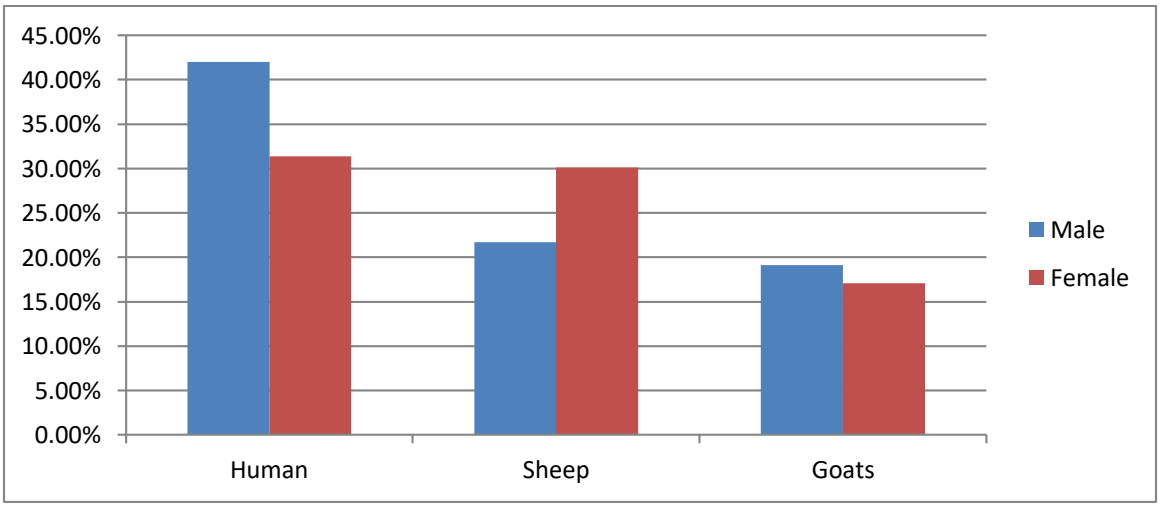

Fig. (3): Prevalence of human, sheep and goat brucellosis by using RBT according to gender

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Human brucellosis in this study had significant different between male and female , brucellosis washigh in male (41.9% positive) than in female (31.4% positive)this was due to more contacts of man than woman with the livestock, this result was in agreement with some studies that reported by (Al-Abbasi, et al., 1991) in Baghdad; (Ali, et al., 1998) in Saudi Arabia. However this result disagree with(Jalat M Shareef, 1999) 35% male and 65% female were positive; (Rasul,D.K. 2012) (30% male and 70% female were positive.

Our results showed asignicant different between male and female of sheep, in male rate was less than in female rate this is in agreement with (Al-Alousi, 2008) 2.54%, 6.44% in Al-Anbar province and disagreement with : (Ahmed et al., , 2013) 13.82%, 9.69% in Al-Anbar province; on the other hand there were not significant different among male and female of goat, this was in agreement with: (Islam, Samad and Rahman, 2012) 3.33%, 3.93% in Bangladesh; (Yesuf *et al.*, 2011) 1.4%, 1.68% in South Wollo, Ethiopia; respectively.

Prevalence of Brucellosis according to age group:

Among the prevalence of sheep and goat brucellosis according to other age group, it was found that the high seroprevalence rate (19.2%) was at 1-3 years old with significant differences ($p \leq 0.05$) as comparison with the low seroprevalence rate (6.25%)that was recorded at <1 year old (Table 5 and fig. 4):

Sero-positivity of human brucellosis in age group:

The results of the present study showed (Table 5, Figure 4) that the highest percentage 17(51.5%) from 33 human sera of sero-positivity occurred among age group (21-30) year and the lowest among the age group (11-20) year.

Table (4): Prevalence of human brucellosis by using RBPT according to age group

Age	No of sample	No. of positive	Rate of positivity
11-20 year	9	2	22.2%
21-30 year	33	17	51.5%
31-40 year	21	9	42.9%
41-50 year	22	7	31.8%
51-60 year	28	7	25%
Total	113	42	37.2%

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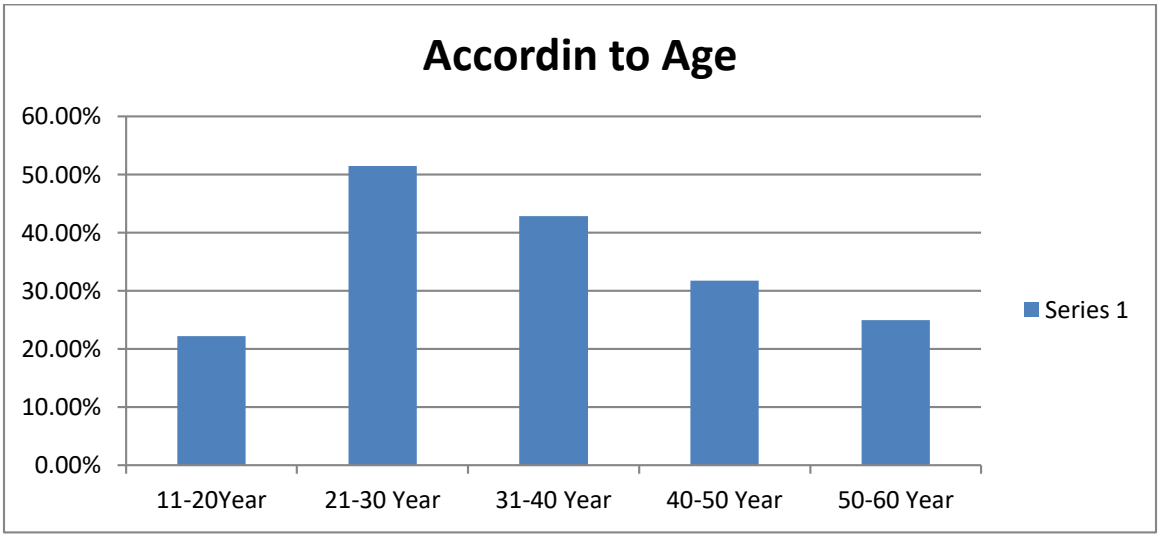

Fig. (4): Prevalence of human, brucellosis by using RBT according to age group.

The present study results illustrate that the highest percentage human brucellosis 51.5% at 21-30 year old in age by using RBT, this was due to that people in this age group furthermore contact with a livestock and more dairy product consumer. In addition to that, this age is more suitable to be a herdsman; therefore this specific occupation has much more contacts with infected animals especially during abortion in which they try to take out the aborted fetuses using the hand without any disinfectant technique. Consequently, the disease will be transmitted to another person by contact or during husbandry process. On the other hand the low percentage of human brucellosis in age group11-20 and 50-60years. This result is in agreement with: (Rasul, et al., 2012) in Erbil city 31.3%for 21-30 age group and 2.6% for 51-60 age group. Also in agreement with results was reported by Al-Freihi (1988) in Saudi Arabia.

Sero-positivity of sheep and goat brucellosis in age group:

The results revealed that the highest percentage 18(36.7%) from 49 sheep sera sample of sero-positivity occurred among age group (4-5) year and the lowest among the age group (2-3) year of the present study which was showed in (table 6, Figure 5)

On the other hand the highest rate of goat infection at 3-4 year old 8 (27.7) sero positive among 30 samples, and the lowest rate at age 1-2years old (table 6, Figure 5)

Table (6): Prevalence of sheep and goat brucellosis by using RBPT according to age group.

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Type of livestock	Age	No of sample	No. of positive	Rate of positivity
Sheep	1-2year	16	3	%18.8
	2-3 year	51	7	% 13.7
	3-4 year	43	15	%34.9
	4-5 year	49	18	%36.7
	above 5 year	40	13	%32.5
Total	1-above 5 year	199	56	%28.1
Goat	1-2year	23	2	%8.7
	2-3 year	67	12	%17.9
	3-4 year	30	8	%27.7
	4-5 year	39	6	%15.4
	above 5 year	23	4	%17.4
Total	1-above 5 year	182	32	%17.9

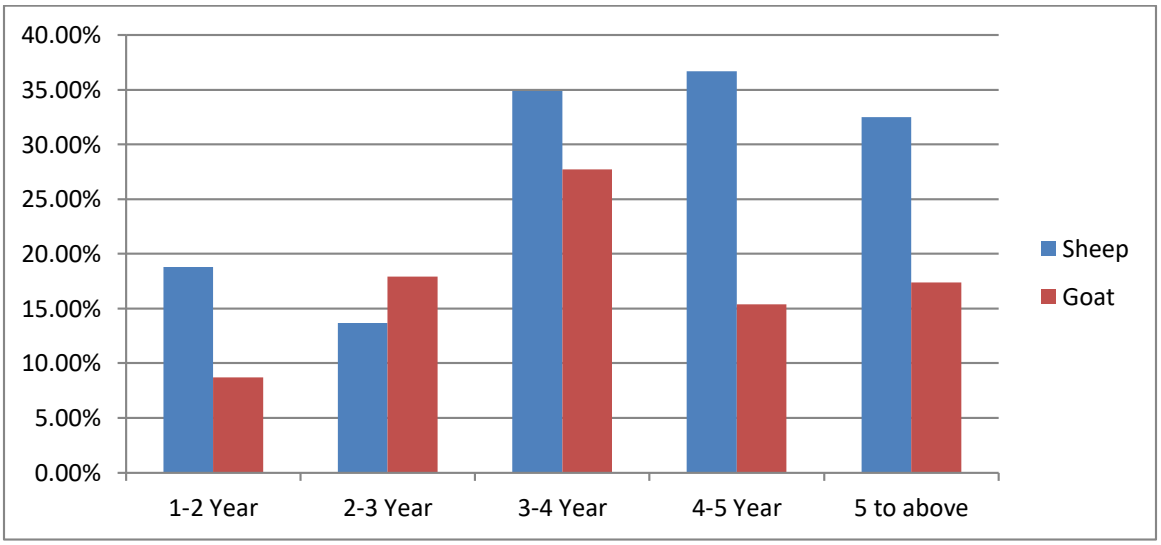

Fig. (5): Prevalence of sheep and goat brucellosis by using RBT according to age group.

The high results of seropositivity shown in a mature age group more than 3 years old, which was above from 30%. Corbel et al., (2006) showed that the prevalence of infection is usually related with age, possibly due to frequent introduction to organism and younger were almost resistant to disease. Furthermore, the animals which are sexually matured are usually susceptible to the brucellosis than non-mature ruminants of either sex(Radostits *et al.*, 2007).

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Results were agreed with (Ferede *et al.*, 2011)who stated high prevalence 1.26% in >1 year old animals in Bahir Dar, Ethiopia; (Adugna, Tessema and Keskes, 2013)who recorded high serosurvey 13.2% in >2 years animal in Afar National Regional state, Ethiopia, while disagreed with (Tesfaye *et al.*, 2012) who found high prevalence 6.5% in young animals in Adamitulu-Jido-Kombolcha district, Ethiopia.

Type and concentration of anti-Brucella antibodies using Enzyme Linked Immunosorbent Assay (ELISA):

Ninety serum samples that having positive, determined by Rose Bengal Test were selected from all our 130 positive samples, and examined for the type of anti-immunoglobulins (Anti- IgM, Anti-IgG) using ELISA. The result showed that the total number of positive cases (IgM and IgG) using ELISA technique were 42(46.7%), 8(19.1 %) have positive for IgM with IgG ,among which11 (26.2%) were of IgM type and 23 (54.8%) were of IgG type. Statistical analysis showed that Anti-IgG type were significantly higher than Anti-IgM type. The concentrations of IgM and IgG for each serum sample determined by ELISA are presented in Table (7).

As only 90 cases under went ELISA, if we it was require to find out the number of sero-positive cases among total 130 Rose Bengal positive cases, it was about 42 cases from 90(46.7 %) were positive. To estimate the total number of sero-positivity by ELISA we used statistical analysis, thus the total percentage of sero-positivity by ELISA was 46.9% (61 of 130 Rose Bengal positive cases)

Table (7) Results of positive anti-brucella IgM and anti-Brucella IgG performed by ELISA technique among RBPT positive cases.

Total examined	Positive				Total Positive		Negative	
	IgM		IgG					
	No.	%	No.	%	No.	%	No.	%
Human	9	45%	11	55%	20	47.6%	22	52.4%
Sheep	11	45.8%	13	54.2%	24	42.9%	32	57.1%
Goat	8	47.1%	9	52.9%	17	53.1%	15	46.9%
Total	28	45.9%	33	54.1%	61	46.9%	69	53.1%

In the present study the seroprevalence of brucellosis from total sample were 12.4% (61/494) This is in agreement with results of: (Godfroid *et al.*, 2011)in the Nowrway and (Seleem, et al., 2010) in USA. They had (10%) the

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positive results of iELISA test. While the results of ELISA was disagreed with results of (Rahman *et al.*, 2011) who recorded 2.3% in sheep and 3.15% in goats in five different districts of Bangladesh; and (Esmaeel *et al.*, 2010) who found 25.3% in sheep and 27.5% in goats in Ninevah province; (Jabary and Al-samarraee, 2015) found (14.46 %) of ELISA results in samarrah,Iraq.

Efficiency of 2-Mercaptoethanol test for the determination of classes of anti-Brucella antibodies:

2Mercaptoethanol test was used to determine the type, number and percentage of anti-IgG and IgM antibodies in the sample serum. The results are presented in table (10) they showed that among 130 samples of RBPT positive cases, 105(80.1%) had IgG antibodies and 59 (45%) had IgM antibodies.

Table (8): Number and percentage of anti-brucella IgM and anti-Brucella IgG performed by 2ME test among RBPT positive case

Total examined	Positive				Total Positive		Total Negative	
	IgM		IgG					
	No.	%	No.	%	No.	%	No.	%
Human	15	%35.7	34	80.9%	27	%64.3	15	%35.7
Sheep	30	%53.6	50	89.2%	26	%46.4	30	%53.6
Goat	14	%43.7	31	73.8%	18	%56.3	14	%43.7
Total	59	%45.4	105	80.1%	71	%54.6	59	%45.4

The differences found in the results obtained by RBT and 2ME were essentially due to the varying levels of sensitivity and specificity (Katayoun H. B., 2012). RBTP test has been accepted as a common test for use in human and all animal species. This test is a simple, rapid and useful test for primary serological detection, but false positive and false negative can occur because cross-reacting antibodies may be present (Al Dahouk, et al., 2013). Rather than this, RBTP gives a positive result with a presence of IgG and IgM, it does not distinguish between recent infections and old infection. Therefore, samples were treated with 2ME solution by mixing v/v together and incubation for 30 min, after which RBTP was repeated. If results are positive it mean that the infection has occurred recently because 2ME solution due to depilation IgM and remaining IgG. Therefore RBTP, after using 2ME, has been found to be a more sensitive and more specific test for the detection of Brucella antibodies and has

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been recommended to be a suitable test for the diagnosis of acute brucellosis. Katayoun et al. found the same result.

Comparison between ELISA and 2-ME technique for detection the class of anti-brucella antibodies:

The results of comparison between ELISA and 2ME for detecting anti-brucella antibodies were presented in table (9) and figure (6) . They showed that when using both 2ME and ELISA test the number of IgG antibodies were significantly higher than the IgM antibodies.

Table (9): Number and percentage of anti-brucella IgM and anti-Brucella IgG performed by ELISA and 2ME test among RBPT positive case

Total examined	Tests	Positive				Total Positive	
		IgM		IgG			
		No.	%	No.	%	No.	%
Human	ELISA	9	45%	11	55%	20	47.6%
	2ME	15	35.7%	34	80.9%	27	64.3%
Sheep	ELISA	11	45.8%	13	54.2%	24	42.9%
	2ME	30	53.6%	50	89.2%	26	46.4%
Goat	ELISA	8	47.1%	9	52.9%	17	53.1%
	2ME	14	43.7%	31	73.8%	18	56.3%

The present study shown that the percentage of IgG was more than the ratio of IgM, this result was in agreement with (Al-Hankawe and Rhaymah, 2012)that they studied comparison between ELISA and other serological tests for diagnosis of brucellosis in sheep in Ninevah Province .However it does not agreed with (Rasul, et al., 2012) human seroprevalence of brucellosis in Erbil city..

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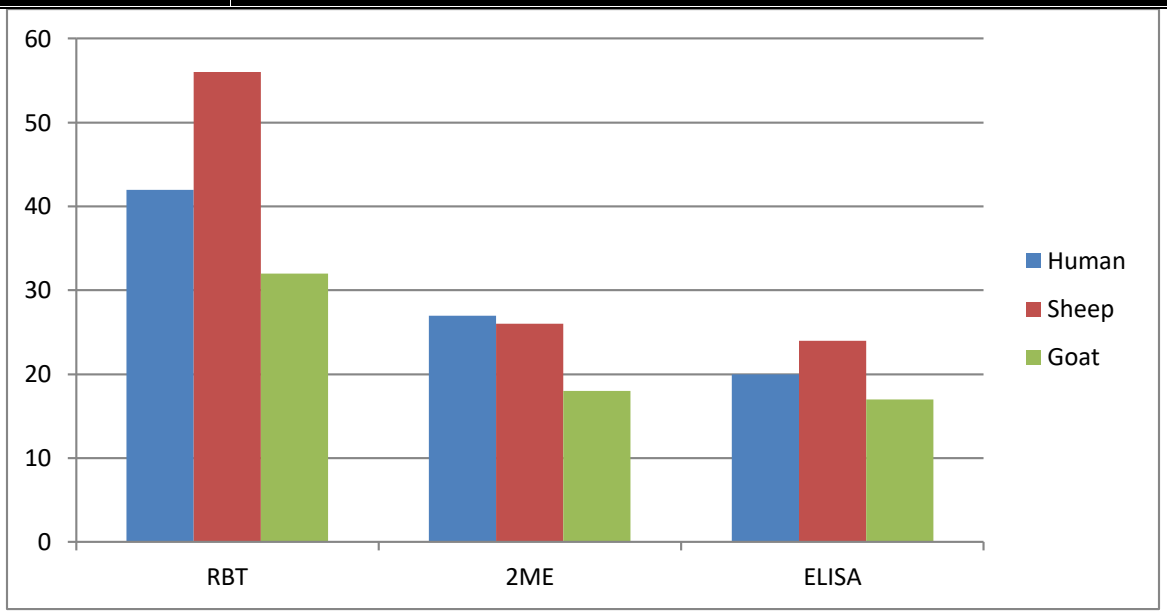
Table (10): Comparison of Serological tests for human, Sheep and goat brucellosis.

Tests	Human		Goat		Sheep		Total	
	No.	%	No.	%	No.	%	No.	%
Rosbengal	42	8.5%	32	6.5%	56	11.3%	130	26.3%
2ME	27	5.5%	18	3.6%	26	5.3%	71	14.4%
ELISA	20	4.1%	17	3.4%	24	4.9%	61	12.4%



Fig(6): Comparison of Serological tests in human, sheep and goat brucellosis.

In the present study, three serological methods were used; Rose Bengal plate test (RBPT), 2ME, and ELISA. The RBT is still the most common serological test that is widely used for diagnosis of Brucella infections depending on the agglutination of colored killed Brucella organisms by specific antibodies present in the blood (Chachra *et al.*, 2009). In this study, RBT detected anti-Brucella antibodies in human also in both sheep and goat with history of abortion and reproductive problems 130 (26.3%) from 494 sera. This result was in agreement with (Rahman et al., 2014)and(Habimana and Nishimwe, 2015). Thus RBPT was decided to be used as a presumptive test due to its high sensitivity(Amin, et al., 2012). (Puran Chand and sharma, 2004)preferred the use of ELISA in comparison to RBT and STAT for assessing the situation of brucellosis in cattle to have better results because chances of non-detection of an infected animal in ELISA are minimum. For this purpose, the ELISA, 2ME and RBT were the serological test used in this study by which the positive reactors of brucellosis of all samples (human and animals with history of abortion and reproductive problems) were 61 (12.4%), 71(14.4%) and 130 (26.3%) respectively. It

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was found that all positive sera for ELISA were also positive for RBT, this result agreed with (Godfroid *et al.*, 2005)and (Seleem, Boyle and Sriranganathan, 2010) Comparing the positive results of ELISA test (10%) with those of RBPT (22%) in this study, it appears that 59sera samples were positive for RBT but negative to ELISA.

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