# Detection of *Brucella* antibodies of sheep and goats by using two serological tests in Al-Sulaimanya governorate

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Accepted: 6/4/2015

## Summary

A research was carried out to detect *Brucella* antibodies of sheep and goats in Al-Sulaimanya governorate by using modified Rose Bengal test and indirect ELISA. A total of three hundred and eleven (311) whole blood samples (160 sheep and 151 goats) were collected randomly from eight different regions in Al-Sulaimanya governorate from unvaccinated flock with different ages. A total percentages of positive result by using modified Rose Bengal test was 14.46% (20% in sheep and 8.6% in goats), while by using indirect ELISA was 27.6% (35.2% in sheep and 19.2% in goats) with significant ($P<0.05$) differences. It revealed that rates to modified Rose Bengal test were 14.34% in female and 10.09% in males while to indirect ELISA 26.35% in female and 33.9% in males in sheep and goats, In conclusion of this study the high seroprevelence was at 1-3 years 19.2% and >3 years 33.96% according to modified Rose Bengal test and indirect ELISA, respectively.

**Keywords: *Brucella* – antibodies, Modified Rose Bengal test, Indirect ELISA, Sheep, Goat.**

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## Introduction

Brucellosis is one of the most important zoonotic disease worldwide (1), resulting in serious economic losses and public health issues (2). It is a disease of animals, especially domesticated livestock including sheep and goats, caused by *Brucella* species. Infections in sheep and goats are highly contagious because of the pathogenicity of *Br. melitensis* and close contact between animals in herd (3). The most frequent sign following infection with *Brucella* is abortion (4). Persistent (lifelong) infection is characteristic, by shedding in reproductive and mammary secretions (5). The disease is common in most developing countries (6). In Iraq, brucellosis is the most common and endemic disease (7) which has been recorded for the first time by (8). Serological tests are widely used to detect specific *Brucella* antibody in sera and other body fluids by a variety of techniques (9) which are fast, non-hazardous and more sensitive, which can only indirectly prove *Brucella* infections by high or rising titers of specific antibodies (10) but lack specificity (11). RBPT and ELISA tests are used for diagnosis of a wide range of animal and human diseases (3 and 12). The aim of this study was to detect *Brucella* antibodies of sheep and goat by modified Rose Bengal test and indirect ELISA.

## Materials and Methods

Three hundred and eleven (311) whole blood samples (160 sheep and 151 goats) were collected randomly from the jugular vein of each small ruminant with different ages, from unvaccinated herds, during the period of January-February/2014. Samples were collected from different districts in Al-Sulaimanya governorate (Kalar, Kfry, Chwarta, Chamchamal, Ranya, Halabja, Said Sadiq and Sharazur). The sera were separated by centrifugation of samples at 1500 r.p.m. for 10 minutes, each serum was put in three eppendorf tubes which were properly labeled then stored at -20°C (12) until examined by mRBT* and ELISA** tests for the presence of *Brucella* antibodies. Data were collected and statistically analyzed by using SPSS program.

## Results and Discussion

The result of this research revealed that 45 (14.46 %) and 86 (27.6%) sera were found positive by using (mRBT) and iELISA respectively in eight administrative regions. These differences observed in results obtained by mRBT and ELISA were essentially due to the varying levels of sensitivity and specificity (13). RBT has been accepted as efficient for use in human and all animal species. This test is a simple, rapid and an excellent test for the large-scale screening of sera. False positive and false negative can occur (9). iELISA has

been found to be more sensitive and more specific test for the detection of *Brucella* antibodies and has been recommended to be stable and suitable test for routine diagnosis of brucellosis (14 and 15). According to mRBT, the higher seroprevalence result 12(30%) was found in Chamchamal district with significant differences (P≤0.05) and lower seroprevalence result 1(3.7%) was recorded in Sharazur, while there was a negative result 0(0%) recorded in Said Sadiq district (Table, 1).

**Table, 1: Positivity of sheep and goat brucellosis by using mRBT according to districts.**

| District | No. of sera tested | Positive result | Percentage (%) |
|---|---|---|---|
| Chamchamal | **40** | **12** | **30** |
| Kalar | **49** | **14** | **28.57** |
| Kifry | **65** | **12** | **18.46** |
| Chwarta | **29** | **2** | **6.89** |
| Rania | **30** | **2** | **6.66** |
| Halabja | **32** | **2** | **6.25** |
| Sharazur | **27** | **1** | **3.7** |
| Said Sadiq | **39** | **0** | **0** |
| Total no. | **311** | **45** | **14.46** |

*** Kit manufactured by SPINREACT Co.-Spain**
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According to iELISA test, a higher seroprevalence 22(55%) was found in Cham-chamal district with significant differences (p≤0.05), while lower seroprevalence result 1(3.7%) was recorded in Sharazur district (Table, 2).

**Table, 2: Percentage of infection of sheep and goat brucellosis by using iELISA according to districts.**

| District | No. of sera tested | Positive result | Percentage (%) |
|---|---|---|---|
| Chamchamal | **40** | **22** | **55** |
| Kalar | **49** | **17** | **34.7** |
| Kifry | **65** | **16** | **24.6** |
| Chwarta | **29** | **7** | **24.1** |
| Rania | **30** | **14** | **46.7** |
| Halabja | **32** | **4** | **12.5** |
| Sharazur | **27** | **1** | **3.7** |
| Said Sadiq | **39** | **5** | **12.8** |
| Total no. | **311** | **86** | **27.6** |

The susceptibility of animals to brucellosis depends on many factors including grazing strategy, disease prevalence (16); geographical variability (17); open surface water, sharing of water with other herds, vaccination (18) and population density (number of animals to land area) which is attributed to increased contact between susceptible and infected animals (11). The herd size appears to be a major risk factor for brucellosis compared with other factors, there is a positive association among population density which is attributed to increased opportunity for animals to come in contact with potentially infected flocks during their movements and co-mingling (19) especially during calving or abortion when most of brucellosis contamination occurs (20). The existing traditional husbandry practices of handling multiple species support the spread of brucellosis in the area (21); so the results of mRBT agreed with result obtained by (22) who found 13.4% in Ninevah province and (23) who found 6.8% in goats in Al-Sulaimanya governorate and disagreed with (24) who found 3.08% in sheep and 4.72% in goats; (25) found 1.69% in goats and 0% in sheep; (26) found 25.3% in sheep and 27.5% in goats; and results of ELISA disagreed with (23) who found 9.1% in sheep and 3.9% in goats. According to animal species, the study revealed differences in the infection rates of brucellosis between sheep and goats. Out of 160 sheep sera tested, 32 (20%) were positive significantly differences (P≤0.05), in contrast, 151 goat's sera tested, 13 (8.6%) were positive with mRBT (Table, 3).

**Table, 3: Percentage of sheep and goat brucellosis by using mRBT according to animal species.**

| Species | No. of sera tested | Positive result | Percentage (%) |
|---|---|---|---|
| Sheep | **160** | **32** | **20** |
| Goat | **151** | **13** | **8.6** |
| Total | **311** | **45** | **14.46** |

While according to iELISA out of 160 sheep sera tested, 57 (35.62%) were positive with significant differences (P≤0.05), in contrast, 151 goat sera tested, 29 (19.2%) were positive (Table, 4).

**Table, 4: Positivity of sheep and goat brucellosis by using iELISA according to animal species.**

| Species | No. of sera tested | Positive result | Percentage (%) |
|---|---|---|---|
| Sheep | **160** | **57** | **35.62** |
| Goat | **151** | **29** | **19.2** |
| Total | **311** | **86** | **27.6** |

Both tests; mRBT and iELISA showed that prevalence of brucellosis were higher in sheep than in goats, and a plausible explanation for this finding was difficult. Authors (27) observed that keeping sheep in contact with goats is a risk factor for brucellosis and ovine animals behaviour that get together in parturition or at night (long-term close contact), which increases potential of disease transmission. Goats do not have this behavior. Present results agreed with results obtained by (28, 29 and 30) in percentage of 12.2%, 11.3%; 4.8%, 2.19%; 21.2%, 14.5% in sheep and goats respectively, and disagreed with (31-33) in percentage of 0.94%, 1.41%; 21.1%, 24.6%; 5.71%, 10% in sheep and goats respectively. The Prevalence of sheep and goat brucellosis by using mRBT according to gender revealed no differences in infection rates of sheep and goat brucellosis. Totally among of 258 female animal sera tested, 37 (14.34%) were positive, while on the contrary, where 53 sera samples from male animals tested, 8 (15.09%) were positive with mRBT (Table, 5).

**Table, 5: Percentage of sheep and goat brucellosis by using mRBT according to gender**.

| Species | Female Samples | | | Male Samples | | |
|---|---|---|---|---|---|---|
| | Sera tested | Positive result | Percentage (%) | Sera tested | Positive result | Percentage (%) |
| Sheep | 125 | 24 | 19.2 | 35 | 8 | 22.8 |
| Goat | 133 | 13 | 9.77 | 18 | 0 | 0 |
| Sum | 258 | 37 | 14.34 | 53 | 8 | 10.09 |

Indirect ELISA test according to gender, revealed non-significant differences (P>0.05) in infection rates of sheep and goat brucellosis. Totally among 258 female animal sera tested, 68 (26.4%) were positive, while 53 sera samples from male animals tested, 18 (34%) were positive (Table, 6).

**Table, 6: Percentage of sheep and goat brucellosis by using iELISA according to gender.**

| Species | Female Samples | | | Male Samples | | |
|---|---|---|---|---|---|---|
| | Sera tested | Positive result | Percentage (%) | Sera tested | Positive result | Percentage (%) |
| Sheep | 125 | 43 | 34.4 | 35 | 14 | 40 |
| Goat | 133 | 25 | 18.8 | 18 | 4 | 22.2 |
| Sum | 258 | 68 | 26.35 | 53 | 18 | 33.9 |

The positivity of brucellosis is high in male than in female animals which could probably be due to the fact that most farmers preferred ability to keep a large number of female and few number of male in the flock. So the results agreed with (34 and 35) and disagreed with (36- 38 and 32). According to age group, the positivity of disease by using mRBT found that the high positivity rate (19.2%) was at 1-3 years old with significant differences (P≤0.05) comparison with the low positivity rate (6.25%) recorded at <1 year old (Table, 7).

**Table, 7: Percentage of sheep and goat brucellosis by using mRBT according to age group.**

| Age group (year) | No. of sera tested | Positive result | Percentage (%) |
|---|---|---|---|
| <1 year | 80 | 5 | 6.25 |
| 1-3 years | 125 | 24 | 19.2 |
| > 3 years | 106 | 16 | 15.09 |
| Total | 311 | 45 | 14.46 |

The high positivity rate (33.96%) by iELISA was at >3 years old with significant difference (P≤0.05) in comparison with the low seroprevalence rate (16.25%) was recorded at <1year old (Table, 8).

**Table, 8: Percentage of sheep and goat brucellosis by using iELISA according to age group.**

| Age group (year) | No. of sera tested | Positive result | Percentage (%) |
|---|---|---|---|
| <1 year | 80 | 13 | 16.25 |
| 1-3 years | 125 | 37 | 29.6 |
| > 3 years | 106 | 36 | 33.96 |
| Total | 311 | 86 | 27.65 |

There was a significant association between age and the positivity of brucellosis in the study. Seropopositivity was observed high in animals which were sexually mature than immature; in *Brucella* infection, positivity increases with age, probably because of greater exposure to infection and younger animals are usually resistant to infection (3). Moreover, sexually mature animals are more prone to the infection than sexually immature animals of either sex. This is related to the fact that sex hormones and meso-erythritol (in male testicles and seminal vesicles) and erythritol in female allantoic fluid stimulate the growth and multiplication of *Brucella*

organisms and tend to increase in concentration with age and sexual maturity (39 and 40). This result agreed with those (24, 34, 36 and 38) and disagreed with the results obtained by (41) who obtained 6.5% in young animals and 3.1% in mature animals.

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## الكشف عن اضداد جرثومة البروسيلا في الاغنام والمعز باستعمال فحصين مصليين في محافظة السليمانية

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**الخلاصة**

هدفت الدراسة الى معرفة مدى الإصابة المصلية لأضداد البروسيلا للإغنام والمعز في المحافظة السليمانية باستعمال عدتي الروز بنغال المحورة (mRBT) وفحص الألايزا غير المباشر (iELISA). من مجموع (311) عينة دم (161 عينة للإغنام و150عينة معز) جمعت عشوائياً من ثمانية مناطق مختلفة من محافظة السليمانية لقطعان غير ممنعة بلقاح البروسيلا وبمختلف الأعمار. ظهرت نسبة الاصابة 14.46% (20% للأغنام و8.6% للمعز) و27.6% (35.2% بالأغنام و19.2% بالمعز) وكانت مهمة احصائياً بمستوى ($P< 0.05$) باستعمال فحصي الروز بنغال المحور والألايزا غير المباشر على التوالي، وقد بلغت نسبة الإصابة في الإناث 14.34% والذكور 10.09% وكذلك 26.35% في الإناث و33.9% في الذكور باستعمال فحصي الروز بنغال المحور والألايزا على التوالي. نستنتج من الدراسة أن أعلى مستوى للإنتشار المصلي 19.2% ظهر بعمر 1-3 سنة وكذلك كان 33.96% بعمر أكثر من ثلاث سنوات للمدتين أعلاه على التوالي.

**الكلمات المفتاحية: أضداد جرثومة البروسلا، روز بنغال المحورة mRBT، فحص الألايزا غير المباشر iELISA، الأغنام، المعز.**