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Seroprevalence of Brucellosis in Small Ruminants Assayed by The Rose Bengal Test, Al-Jabal Al-Akhdar Libya

Nawara M. B. Eissa[1]*, Aiman M. Arhaim[2], Khaled M. A. Hussin[1] and Abdul Hafeez. H. Nimir[1]

[1] Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Libya.
[2] Department of Clinical pathology, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Libya.

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Abstract: This study aimed at determining the prevalence of anti- *Brucella* antibodies in small ruminants in Al- Jabal Al- Akhdar area, Libya. Nine regions were selected for the investigation (Al- Goba, Al- Wasata, Side Kahled, Lamloda, Al-Hesha, Marawa, Al-Gagab, Gandola and Ain Mara). Seroprevalence was assayed using the Rose – Bengal Plate Test (RBPT). Four hundred blood samples were collected randomly from 247 sheep and 153 goats with a history of abortion and reproductive disorders, during the period from January 2015 to June 2016. Approximately 10 ml blood sample was taken from each animal, in vacutainers. Serum samples were separated and subjected to examination by the RBPT. Samples showing visible agglutination within 4 mins. were regarded as positive for anti- Brucella antibodies. Data were analyzed statistically by the Chi- square test using the SpSS software, at $p \leq 0.05$ level of significance. Out of the 400 ovine and caprine sera tested, 125 (38%) were positive for anti – *Brucella* antibodies by the RBPT (Table 3). The rate of seropositivity was higher in goats (69.3%) than in sheep (18.6%) (Table 2). There were variations in seroreactivity from different regions. For instance, sera from Al- Hesha and Gandola exhibited 100% positivity, whereas those from both species in Al- Gagab were remarkably sero-negative (0%) (Table 3). Striking differences were shown by the sera from Gandola and Ain- Mara. Where all the caprine sera from Gandola were positive for anti – *Brucella* antibodies, all the 18 sera from Ain- Mara were serologically negative. Serum reactivity from both goats and sheep in other regions ranged between 60 and 83.3% in goats and 11.5 and 23.3% in sheep (Table 3). It can be concluded that the prevalence of anti- *Brucella* antibodies is high in small ruminants of Al- Jabal Al- Akhdar, Libya and may indicate a possible existence of Brucellosis in goats and sheep.

Keywords: Rose Bengal test, Small ruminants, brucellosis.

INTRODUCTION

Brucella is a Gram - negative facultative intracellular organism responsible for a variety of disease conditions and has a zoonotic significance. Brucellosis is caused by bacteria of the genus *Brucella* and is reported worldwide causing abortion, infertility, retained placenta, endometritis in females and to a smaller extent, orchitis, and infection of the accessory sex glands in males (Mustafa *et al.,* 2011). Ten species are recognized within the genus *Brucella*. There are six 'classical' species: *B. abortus, B. melitensis, B.*

***Corresponding Author:** Nawara M.B. E. nawara.belgasim@omu.edu.ly, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Al-Bayda, Libya

suis, B. ovis, B. canis and *B. neotomae* and other four species have been recognized more recently (Atluri V.L. *et al.,* 2011). Brucellosis is a worldwide re-emerging zoonosis that causes severe disease in humans, with non-specific clinical signs affecting numerous organs (Seleem *et al.,* 2010).

Contact with infected animals, ingestion of contaminated animal products and handling of Brucella isolates in laboratories are risk factors. Brucellosis in livestock and humans is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean. *B. melitensis* is particularly common in the Mediterranean basin, and it has also been reported from Africa, India, and Mexico (Kassahun *et al.,* 2010). Ovine brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *B. ovis*, while classical brucellosis is caused by *B. melitensis* and constitutes a major public health threat equal to caprine brucellosis (Acha and Szyfres 2003). About 500,000 new human cases of brucellosis are reported annually worldwide making it the most common zoonosis (Seleem *et al.,* 2010). Status of the disease in small ruminants in a country can be known only through effective sero-monitoring using serological tests and random sampling methods for the disease. The economic importance of brucellosis in sheep and goats requires the use of sensitive and rapid diagnostic methods.

Diagnosis of *B. ovis* and *B. melintensis* infection is based on clinical examination, serological tests, biotechnological techniques, and cultural isolation (Webb *et al.,* 1980). The laboratory isolation and identification of *Brucella* organisms are the most reliable methods of diagnosis but are not always successful. And they are not practicable in terms of time and labor for field and laboratory personnel when large numbers of animals are involved and also cumbersome and pose a great risk to the laboratory personnel. The biotechnological procedures require trained persons and the establishment of advanced laboratories. Consumption of unpasteurized milk and milk products from cows, small ruminants or camels is considered to be the main route of infection as well as an occupational hazard (Almuneef *et al.,* 2004). In the North African region, as in sub-Saharan countries, social and economic factors play a major role in the spread of brucellosis (Makita K *et al.,* 2008). Brucellosis is considered to be endemic in Libya (Pappas *et al.,* 2006), although little information is available; previous studies are limited to food-producing animals such as cattle and goats (Gameel *et al.,* 1993) and reports of human brucellosis in Libya are limited to a few cases (Tiller *et al.,* 2009).

MATERIALS AND METHODS

Study area

A total of 400 blood samples were randomly collected from nine different regions of Libya (Al-Goba, Al- Wasata, Side Kahled, Lamloda, Al-Hesha, Marawa, Al-Gagab, Gandola and Ain Mara); 247 samples from sheep and 153 samples from goats (Table 1). The samples were collected during the period from January 2015 to June 2016.

Serum sample collection and submission

Approximately 10 ml of blood was collected from each animal using a Vacutainer and needle. The sample containers were tilted horizontally, overnight at room temperature to allow clotting. Serum from each animal was decanted into a single sterile cryogenic vial, labeled and transported to the laboratory of clinical pathology, Omer Almukter University, for investigation. The sera were stored at −20°C until tested.

Samples

A total of 400 serum samples of small ruminants comprising 247 from sheep and 153 from goats (Table 1), having the history of abortion and reproductive disorders like endometritis, retention of placenta, infertility and repeat breeding, were randomly collected from nine different locations. All the serum samples were tested for the presence of Anti-*Brucella* antibodies by using the serological test Rose Bengal Plate Test (RBPT).

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Table (1): Samples distribution from different regions in Al- Jabal Al- Akhdar , Libya.

Animal species	No. samples	Regions								
		Al- Goba	Al- Wasata	Sidi Khaled	Lamluda	Al-Heisha	Mrawh	Al-Gagab	Qandula	Ain Mara
Sheep	247	139	23	0	30	6	26	5	0	18
Goats	153	45	30	10	9	6	20	0	8	25
Total	400	184	53	10	39	12	46	5	8	43

RBPTprotoc

The RBPT (Cromatest, Spain) was performed according to the procedure described by Alton *et al.,* .(1988). To perform the test, antigen and serum were thawed and then brought to room temperature. The bottle containing antigen was shaken well to ensure homogenous suspension. Then, one drop (0.03 ml) of serum sample and one drop of antigen were put on the same slide using different micropipettes and mixed thoroughly using a spreader. The slide was rotated for 4 min. and observed immediately. Then after further 4 min. for results, a result was considered positive when there was noticeable agglutination after 4 min.

Data analysis

All data were analysed by Chi–square test, using the SPSS statistical software. All statistical tests were conducted at $p < 0.05$ level of significance.

RESULTS

The sero-prevalence of brucellosis in small ruminants is summarized in (Table 2). A total of 400 serum samples (from 247 sheep and 153 goats) were collected and tested. Of the 400 ovine and caprine sera tested, 152 (38%) were positive for Anti-*Brucella* antibodies by RBPT. Rates of seropositivity were higher in goats (69.3 %) than in sheep (18.6%) (Figure 1). Consequently, the incidence rate of brucellosis based on RBPT showed a high percentage of positive reactors in the overall prevalence of *Brucella* seropositivity among goats.

Table (2): Prevalence of Anti-*Brucella* antibodies in small ruminants species assayed by the Rose Bengal test, Libya.

Animal species	Animals tested	Seropositive animals	Proportion of posit animals
Goats	153	106	69.3 %
Sheep	247	46	18.6 %
Total	400	152	38%

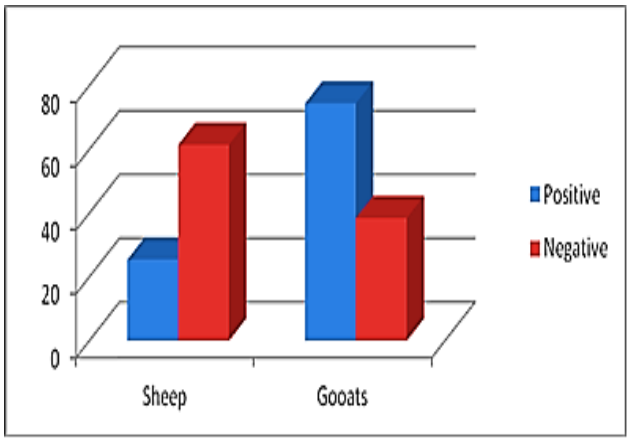

Figure (1): Prevalence of Anti-Brucella antibodies in small ruminants species assayed by the Rose Bengal test, Libya.

Test sera from Al-Hesha and Gandola municipalities showed the highest seropositivity (100%), whereas test sera from both species in Al-Gagab exhibited remarkable seronegativities by the RBPT (0%). It is worth mentioning that only 5 ovine samples from Al- Gagab were collected and investigated. Striking differences were exhibited by the caprine sera from Gandola and Ain- Mara. Where the caprine samples from Gandola showed 100% seropositivity, all the 18 ovine sera from Ain-Mara were serologically negative compared to 52% positivity by the 25 caprine samples from

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the same region. It is also noticeable that the 6 caprine serum samples from Al-Hesha were 100% positivity whereas the 6 ovine samples from the same area gave 50% seropositivity. Serum reactivity from both goats and sheep from other regions ranged between 60 - 83.3% in goats and 11.5 - 23.3% in sheep (Table 3) (Figure 2).

Table (3): The incidence rate of Brucellosis among small ruminants at different regions in Al- Jabal Al- Akhdar , Libya.

Region	Number of samples		Positivity of samp		Animals Species					
					Goats (153)			Sheep (247)		
	No	%	No	%	No	Pos	%	No	Pos	%
Al- Goba	184	46%	56	30.4%	45	28	62.2%	139	28	20.1%
Al- Wasata	53	13.3%	30	56.6%	30	25	83.3%	23	5	21.7%
Side Kahled	10	2.5%	7	70%	10	7	70%	0	0	0%
Lamloda	39	9.6%	14	35.9%	9	7	77.8%	30	7	23.3%
Al-Hesha	12	3%	9	75%	6	6	100%	6	3	50%
Marawa	46	11.5%	15	32.6%	20	12	60%	26	3	11.5%
Al-Gagab	5	1.3%	0	0%	0	0	0%	5	0	0%
Gandola	8	2%	8	100%	8	8	100%	0	0	0%
Ain Mara	43	10.8%	13	23.3%	25	13	52%	18	0	0%
Total	400	100%	152	38%	153	106	69.3%	247	46	18.6%

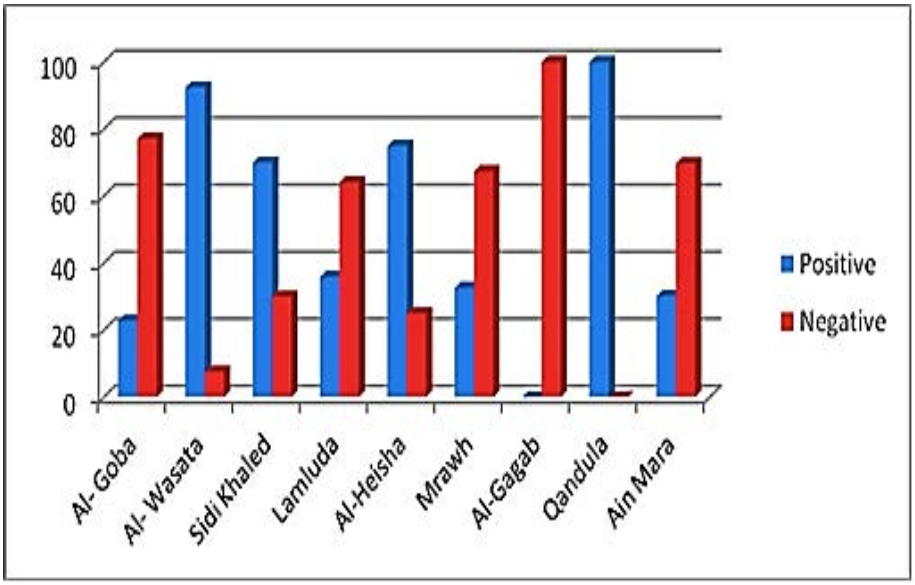

Figure (2): The incidence rate of Brucellosis among small ruminants at different regions in Al- Jabal Al- Akhdar , Libya.

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DISCUSSION

The prevalence of brucellosis observed in small ruminants in Al-Jabal Al-Akhdar in Libya was lower than most values reported in other African countries. This may be attributed to the low level of intensification, breed differences, flock size and composition, or the tests used to make the diagnosis. Brucellosis is a worldwide zoonotic disease that is recognized as a major cause of heavy economic losses to the livestock industry and poses a serious human health hazard (Ocholi *et al.,* 2005). In the present study, Table (2) shows the incidence of brucellosis among small ruminants in Al-Jabal Al-Akhdar in Libya by using RBPT. The incidences of brucellosis were 69.3 % and 18.6 % in goats and sheep respectively.

A local serological survey at the Al Jabal al Gharbi University in the western mountains region in 1997 found that 8.5% of sheep and 28.4% of goats were positive for brucellosis (Elarbi 1997). The obtained result was nearly similar to that recorded by Samaha *et al.,* .(2009) but lower than that reported by Ali and Mahdey.(2010), Holt *et al.,* .(2011) and DaSilva *et al.,* .(2014). A higher seroprevalence in goats than in sheep has also been described by other authors (Gargouri et al., 2009), Prevalence values between two- and fourfold higher in goats have been described in Eritrea (Omer *et al.,* 2000), East Morocco, Tunisia and Egypt (Benkirane 2006) and Nigeria (S.I.B. *et al.,* 2006), and between one and two-fold higher in Sudan, the United Arab Emirates (Benkirane 2006) and in Kenya (Ndarathi and Waghela 1991). In other countries, a higher prevalence has been detected in sheep. For example, Somalia (Andreani *et al.,* 1983), Jordan (Benkirane 2006) and Oman (Ismaily *et al.,* 1988). Programs and control measures have been undertaken in many countries in North Africa and the Near East (e.g. Egypt and Kuwait) (Samaha *et al.,* 2009). However, underreporting and under diagnosis of other food-borne pathogens are problems around the Mediterranean (Gargouri *et al.,* 2009), particularly in North African countries where communication with local authorities is problematic and most of the available information is unpublished or limited to seminars and workshops (Refai 2002).

Generally, goats are more susceptible to *Brucella* infection than sheep, and this could be partly due to the fact that sheep excrete the organism for shorter periods compared with goats. This may reduce the potential for spread of the disease within and between sheep flocks (Radostits *et al.,* 2000). The prevalence and severity of disease may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factors (Amin *et al.,* 2005). Another interesting result of our study is that individual seroprevalence was significantly higher in goats than in sheep. Our results are consistent with others reported by Coelhoa and Coelho.(2013) who found that goats are more susceptible to the infection than sheep. However, these results are in contrast with (Reviriego *et al.,* 2000). In addition, the results from this study indicate that Brucellosis is more prevalent in Gandola (100%) followed by Al-Hesha (75 %) than in other investigation districts (Table. 3).

The difference in infection rates between different districts in Al-Jabal Al-Akhdar governorate may be due to the difference in applied management in each area, failure, or absence of vaccination program in some herds. Differences between the prevalence of Brucellosis obtained in this study and those obtained by other authors may be attributed to various factors such as the season during which this study was performed, the area from which animals were examined, as well as the evolutionary changes in the animal husbandry which affect the rate of exposure and the different serological tests used confirmed by bacterial isolation.

CONCLUSION

Brucellosis is still a major disease of worldwide distribution. There are many factors involved in both human and animal brucellosis that make the control and eradication of this disease an

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important challenge. We conclude that in Al-Jabal Al-Akhdar in Libya, Brucellosis seroprevalence is high in small ruminants. Our data highlight the need for further researches, including the isolation and characterization of the causative agents, reliable epidemiological studies and the need to implement a transparency policy and effective control measures in Libya. Today, we have very powerful tools to fulfill the requirements: excellent serological methods, very effective immunogens and an overall knowledge of the pathogenesis of this disease.

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الإنتشار المصلي لمرض البروسيلا في المجترات الصغيرة بواسطة اختبار روز بينقال في منطقة الجبل الأخضر- ليبيا

نواره بالقاسم عيسى[1]*، أيمن محمد ارحيم[2]، خالد مسعود حسين[1] وعبد الحفيظ حسن النمر[1]

[1] قسم طب وجراحة الحيوان، كلية الطب البيطري، جامعة عمر المختار، البيضاء، ليبيا.

[2] قسم التشيخصات الإكلينيكية، كلية الطب البيطري، جامعة عمر المختار، البيضاء، ليبيا

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المستخلص : هدفت هذه الدراسة إلى استقصاء احتمال وجود الأجسام المضادة للبكتريا المسببة لمرض البروسيلا في المجترات الصغيرة في منطقة الجبل الأخضر – ليبيا. اختيرت تسعة مواقع لإجراء الدراسة، هي: (القبة، الوسيطة ، سيدي خالد ، لملودة ، الهيشة ، مراوة ، القيقب ، قندولة وعين مارة). استخدم إختبار روز بنقال بالطبق لإجراء الدراسة. جمعت أربعمائة عينة دم عشوائية من 247 رأس من الأغنام و 153 رأس من الماعز، لها تاريخ مرضي مثل الإجهاض وإضطرابات الجهاز التناسلي وذلك خلال الفترة من يناير 2015 حتي يونيو 2016. أخذت حوالي 10 مل من الدم من كل الحيوانات، وخضعت للإختبار بواسطة اختبار روز بنقال. اعتبرت عينة المصل التي أعطت تراصا (تلازنا) خلال 4 دقائق موجبة لوجود الأجسام المضادة لبكتريا البروسيلا. استخدم اختبار "Chi – Square" لتحليل البيانات. من مجموع 400 عينة تم اختبارها أظهرت 152 عينة بنسبة 38% نتائج موجبة مع ملاحظة أن معدل الإيجابية كان أعلى في أمصال الماعز (69.3%) عنها في أمصال الضأن (18.6%). اتضح أن هناك تبايناً في نسبة الإيجابية في الأمصال من مناطق الدراسة المختلفة. مثلا: أظهرت الأمصال من الهيشة وقندولة نسبة 100% إيجابية، وكانت التي جُمعت من القيقب سلبية بنسبة (0%). ظهرت تباينات لافتة للنظر في نتائج الأمصال من قندولة وعين مارة ففي حين أعطت كل الأمصال المعزية من قندولة تفاعلا موجبا يومئ بوجود الأجسام المضادة لبكتريا البروسيلا أعطت كل الأمصال الـ8 من عين مارة تفاعلاً سلبياً. تراوحت نسبة التفاعلية المصلية في كل من الماعز والضأن في المناطق الإخري من 60-83.3 % في أمصال الماعز إلى 11.5-23.3 % في أمصال الضأن. ويستنتج من هذه الدراسة أن هناك احتمالاً لوجود الأجسام المضادة للبكتريا البروسيلا بمعدلات عالية في المجترات الصغيرة في منطقة الجبل الأخضر _ ليبيا ، مما يشير إلى احتمال وجود مرض البروسيلا في الضأن والماعز.

الكلمات المفتاحية: اختبار الروزبنقال، المجترات الصغيرة، مرض البروسيلا (الإجهاض السري).

*نواره بالقاسم عيسى nawara.belgasim@omu.edu.ly : قسم طب وجراحة الحيوان، كلية الطب البيطري، جامعة عمر المختار، البيضاء، ليبيا.