Seroprevalence Study of Brucellosis in Rams of Sulaimani region

Talib Ghaidan Mannt Ali, Faridoon Abdul sattar Mahamed Amin and Rebwar Bahir Ahmed
Department of Surgery and Theriogenology, Department of Clinic and Internal Medicine / College of Veterinary Medicine / University of Sulaimani / Kurdistan Regional Government-Iraq.

E-mail: talib_1960@yahoo.com.
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Summary

The aim of our study is to detect the incidence of Brucella infection in rams in Sulaimani region by using Rose Bengal, 2 Mercaptoethanol and indirect ELISA tests. The following research was conducted on 253 rams in different fields of sheep they were distributed in eight districts, represented by Pebaz (12 ram), Chamchmal (53 ram), Barika (32 ram), Damrkan (27 ram), Qalghanlw (41 ram), Darbasara (26 ram) , Dawye (38 ram) and Bazian (24 ram). Blood samples were collected from jugular vein. All blood samples were proceeds for sera preparation in veterinary teaching hospital / college of veterinary medicine / university of Sulaimani. Out of 253 rams 61 (24.11%) and 192(75.89%) were positive and negative for brucellosis respectively during Rose Bengal test. All rams showed the infection by brucellosis throughout the titer of antibodies. The serological test by 2 mercaptoethanol used for distinguishes the acute and chronic infection by assessment the titer of IgG and IgM antibodies. A percentage of 51(83.61%) and 10(16.39) for acute and chronic infection of brucella respectively were recorded. The results by iELISA manifested rams in all field 42(68.8%) were positive while 19(13.15%) were negative. The research showed 18(29.50%) of rams were manifestation the clinical signs represented by epididymitis, hyperthermia and orchitis. Static analysis revealed there is no significant (P-value<0.05) differences between iELISA and 2ME test. Our conclusion from this study confirmed that this disease of brucella is endemic in province of Sulaimani; in addition the disease is spread in the flock of sheep via sexual contact during mating. Control of the diseases must be implement a global program represented by vaccination as well as transfer of animals and eliminate the infected ram from the herds.

Keywords: Indirect ELISA, Rose Bengal Test, 2mercaptoehanol, Brucella, Rams.

Introduction

Ovine Brucellosis is a common infectious disease. Brucellosis can have a significant impact on the productivity of sheep flock. Infected rams will spread the disease via the ewe. Brucellosis is a bacterial zoonotic infection is amongst the most important diseases, in terms of loss to economy that affects sheep and goat population in the developing countries (1 and 2). It is contagious diseases caused by bacteria of genus Brucella . In places where brucellosis is endemic, humans can get infected via contact with infected animals or consumption of their products, mostly milk and milk products especially cheese made from unpasteurized milk of sheep and goats and rennet from infected lambs and kids. Some species occupational groups including farm workers, veterinarian, ranchers, and meat-packing employees are considered high risk (3). The disease in animals is also called as Bang's disease, Enzootic Abortion and Epizootic Abortion (4). Abortion is the most obvious manifestation in ewes and epididymitis in rams. Infections may also cause stillborn or weak newborn, retained placentas, and reduced milk yield (5). Brucellosis is worldwide in distribution and is more common in countries with poor animal and public health programs. Though it has been eradicated from many developed countries like Australia, Canada, Palestine, Japan, New Zealand and Europe (6 and 7). The disease remains an uncontrolled problem in regions of high

endemic such as Africa, the Mediterranean, Middle East, and parts of Asia and Latin America. Serological tests, Rose-Bengal Test, 2Mercaptoethanol test and ELISA are generally used for the detection of Brucella infection in livestock. Rose Bengal test are used clinically for diagnosis of brucellosis. ELISA has been evaluated for many years for their ability to detect serum antibodies to brucellosis in domestic animals. ELISA for the diagnosis of brucellosis has several advantages when compared with other tests. ELISA results provide an epidemiological tool for investigating the infection status of flocks in places where vaccination has never been practiced (8). The objective of this study was to detect the incidence of brucella infections in rams in Sulaimani region by using serological tests, Rose Bengal test, 2 Mercaptoethanol and indirect ELISA.

Materials and Methods

The study was carried out for a period of one year from (2015 to January 2016) in the Veterinary teaching hospital, Department of Medicine, College of Veterinary Medicine, University of Sulaimani. The research was conducted on 253 rams from different fields of sheep without vaccination suffered from abortion. The fields of sheep were distributed in 8 districts at Sulaimani egion namely; Pebaz, Chamchamal, Barika, Damrkan, Qalghanlw, Darbasara, Dawye and Bazian. The clinical signs of infected rams from Brucellosis were recorded, when ram exhibit several signs represented by; orchitis, epididymitis and hyperthermia. The blood samples were collected aseptically obtained from the jugular vein of all rams. Blood samples were transmitted in a cool box to the veterinary teaching hospital. All blood samples were processed for sera preparation by centrifuge (3000 rmp/1) for 4 minutes and then subjected firstly to Rose-Bengal test in order to identify infected rams. The test of serum samples and Rose-Bengal antigen were kept for one hour at room temperature before the beginning of the test. A result was considered positive when there was any degree of agglutination noticeable and the absence of agglutination was considered as negative. The serum from animals positive by RBPT when showing 40 IU/ml or above titter was considered as positive. Secondly by 2mercaptoethanol then thirdly by indirect ELISA. Rose-Bengal test was performed according to the procedure described by OMEGA DIAGNOSTIC LTD, United Kingdom (2012). ELISA was performed according to the protocol provided by the ELISA kit manufacturer company. The statistical analysis of the data was performed by using the IBM SPSS (9).

Results and Discussion

Brucellosis is a general term used for animal and human infections that is results of several species of the genus Brucella (10). Infection with Brucella is remaining one of the most important and widespread zoonosis, and serological surveillance is essential to its diagnosis and control (11 and 12) In table (1); out of 253 rams 61(24.11%) positive while 192 (75.88%) negative by RBT. While eighteen rams were exhibit clinical signs of brucellosis represented by orchitis, epididymitis and hyperthermia in different livestock. These percentages are considered higher than recorded by (13- 15) which was 5.5% and 6% respectively. Eighteen rams (29.50%) were exhibit clinical signs of brucellosis (orchitis, epididymitis and hyperthermia). Ram epididymitis is caused by non-zoonotic agent Brucella ovis (16). The lower incidence of the former study may be due to good and controlled management practices and screening of male animals for the disease before allow of them for breeding (17).

Table, 1: Result of seroprevalence rate of Anti-Brucellosis Antibody in Rams by Rose Bengal Test (RBT).

Areas	No. of tested Ram	Positive		Negative	
		No.	%	No.	%
Pebaz	12	9	75	3	25
Chamchamal	53	8	15.09	41	84.91
Barika	32	6	18.75	26	81.25

Damrkan	27	8	29.62	19	70.38
Qalghanlw	41	6	14.63	35	85.37
Darbasara	26	7	26.92	19	73.08
Dawye	38	12	31.58	26	68.42
Bazian	24	5	20.83	23	95.83
Total No.	253	61	24.11	192	75.88

Also table (1) showed out of 12 rams 9 (75%), 53(8,15.9%), 32(6,18.75%), 27(8,29.62%), 41(6,14.63%), 26(7,26.92%), 38(12,31.58%), 24(5,20.83%) positive results for Pebaz, Chamchamal, Barika, Damrkan, Qalghanlw, Darbasara, Dawye and Bazian respectively. While the negative result as follow; out of 12 rams (3,25%), 53(41,84.91%), 32(26,81.25%), 27(19,70.38), 41(35,85.37%), 26(19,73.08%), 38(26,68.42%) and 24(23,95.83%) for Pebaz, Chamchamal, Barika, Damrkan, Qalghanlw, Darbasara, Dawye and Bazian respectively.

This investigation was agreed with (18 and 19) .A high prevalence can be determined by RBT because this method has high sensitivity compared to the other diagnostic methods (20). A group of researchers confirmed that the incidence of brucellosis is widespread in sheep herds in Iraq, including the province of Sulaimani, and the proportions of infection was close to (21-23). Poor sanitation, bad management, unawareness to the reproductive health program especially aborted animals, culling of infected rams in flock in addition to environmental situation caused distribution the disease in different region. Ovine epididymitis has been reported in all countries with sheep-breeding tradition. The causative agent can also be isolated from sero-negative and clinically healthy rams (24). Results of antibody titer by rose bangle test in (Table, 2): showed severity of brucellosis as follows; 2(3.28%) for titer 1/40, 9(14.75%) for titer 1/80, 19(31.15%) for titer 1/160, 17(27.87%) for titer 1/320, 12(19.67%) for titer1/640, and 2(3.28%) for titer 1/1280, in all infected rams. The titer of antibodies of brucellosis in rose Bengal test ranged between 1/40 to 1/1280 which can be expressed in international units and correlated well with clinical manifestation of infection (25). The investigation showed that the all rams were infected in Qalghanlw and Dawye through the measurement of the titer of antibodies. In (Table, 2) the titer of antibodies were (16.66%) and (8.33%) for Qalghanlw and Dawye respectively when the titer at level 1/40.

Table,2: Titer of antibody of Brucellosis in Rose Bengal Test.

Area	Positive	**1/40		1/80		1/160		1/320		1/640		1/1280	
	N0.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pebaz	9	0	0	2	22.22	2	22.22	3	33.34	2	22.22	0	0
Chamchamal	8	0	0	1	12.5	3	37.5	1	12.3	3	37.5	0	0
Barika	6	0	0	1	16.66	3	50	2	33.34	0	0	0	0
Damrkan	8	0	0	3	37.5	2	25	2	25	1	12.5	0	0
Qalghanlw	6	1	16.66	0	0	3	50	1	16.67	1	16.67	0	0
Darbasra	7	0	0	1	14.29	2	14.29	2	28.57	2	28.57	0	0
Dawye	12	1	8.33	1	8.33	3	25	4	33.34	1	8.33	2	16.67
Bazian	5	0	0	0	0	1	20	2	40	2	40	0	0
Total No.	61	2	3.28	9	14.75	19	31.15	17	27.87	12	19.67	2	3.28

** Mean all samples were positive.

Detection of acute and chronic brucellosis in rams was done by the test of 2mercaptoethanol for observation the antibody IgG and IgM. Table (3), showed out of 61 cases of brucellosis 51(83.61%) positive reaction while 10(16.39%) negative reaction respectively in all different region. These percentages are agreed with (26 and 27). Obviously, rams are considered carrier as chronic infection and become the main source of transmitted of the brucellosis to the healthy ewes which caused abortion or still birth as

well as infected the human. So 2mercaptoethanol test is a serology test used for distinguishes the acute and chronic infection in rams by assessment of antibodies titer. In our study in table (3) recorded a percentage of 51(83.61%) and 10(16.39) for acute and chronic infection respectively. The results of third test were done by ELISA showed in table (3); were out of 61 cases of brucellosis in rams in all field 42(68.85%) were positive while 19(31.15%) were negative. This percentage were higher than recorded by (28 and 29) which was (53.7% and (10.0%) respectively. The higher seroprevalence of infection in rams is still the result of the lack of implementation of control programs over the years (30).

Table, 3: Seroprevalence of antibody by ELISA and 2ME in Ram.

	Positive Sample by RBT.	Positive ELISA	Positive* 2ME	Negative ELISA	Negative** 2ME
		No. %	N0. %	No. %	No.%
Pebaz	9	6(66.67)	7(77.78)	3(33.33)	2(22.22)
Chamchamal	8	7(87.5)	5(62.5)	1(12.5)	3(37.5)
Barika	6	4(66.66)	6(100)	2(33.34)	0(0)
Damrkan	8	6(75)	8(100)	2(25)	0(0)
Qalghanlw	6	3(50)	4(66.67)	3(50)	2(33.33)
Darbasara	7	5(71.43)	6(85.71)	2(28.57)	1(14.29)
Dawye	12	7(58.33)	10(83.33)	5(41.67)	2(16.67)
Bazian	5	4(80)	5(100)	1(20)	0(0)
Total No.	61	42(68.85)	51(83.61)	19(31.15)	10(16.39)

*Positive serum indicated chronic disease (IgG), ** Negative serum indicated acute disease (IgM).Level of significant (P-value<0.05), the value represents [No. (%)].There is No significant (P-value<0.05\ differences between ELISA and 2ME test. Test Chi- square.

The static analysis revealed there is no significant (P-value<0.05) differences between ELISA and 2ME test. Epizootiological data indicate that the disease has spread through the flocks via sexual contact during natural mating (31-33). The higher percentage of infected rams in present study indicated that the level of implementation of control of the disease is generally considered as lower than in the rest of world. In addition the rams are often introduced without any control and are commonly trade between flocks which further the spread of the infection. Molecular investigation such as ELISA, CFT and GAD in addition to bacteriological isolation should be confirmed for brucellosis infection (34-36). The conclusion from this study that brucellosis is still remaining one of the most important endemic infections in the livestock of sheep in our region and serological surveillance is essential for the diagnosis of infection. Finally, for the control and eradication of the infection in our region the national program should be applied.

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دراسه وبائية مصليه للبروسلا في الكباش لمنطقه السليمانيه

طالب غيدان منت علي ، فريدون عبد الستار محمد امين ، ريبوار باهر احمد
فرع السريريات والطب الباطني/كلية الطب البيطري / جامعة السليمانية /العراق.
E-mail: talib_1960@yahoo.com

الخلاصة

الهدف من الدراسة هو الكشف عن نسبة حدوث حالات الإصابة بالبروسيلا في الكباش في منطقة السليمانية باستخدام اختبارات روزبنكال ، 2ميركابتوايثانول واختبار ELISA غير المباشر. أجري البحث على 253 كبش في حقول مختلفة من الأغنام التابعة الى ثماني مناطق ، وتمثلت Pepaz (12 كبش) ،Chamchamal (53 كبش) ، Barika (32 كبش) ،Damarkan (27 كبش) ، Qalghanlw (41 كبش) ، Darbasara (26 كبش) ، Dawye (38 كبش) وBazian (24 كبش). تم جمع عينات الدم من الوريد الوداجي لكباش. تم تحضير امصال الدم لجميع العينات في المستشفى البيطري التعليمي / كلية الطب البيطري / جامعة السليمانية. من مجموع 253 كبش كان نسبة الاصابة 61 (24.11 ٪) و 192 (75.89 ٪) موجب وسالب لمرض البروسيلات على التوالي من خلال اختبار Rose Bengal. وأظهرت النتائج ان جميع الكباش مصابة بعدوى مرض البروسلا من خلال قياس معاير الاجسام المضادة. كذلك بين نتائج البحث حالات الاصابة الحادة والمزمنة للمرض.عن طريق اختبار Mercaptoethanol2 تم كشف الاصابة لحالات الحادة والمزمنة عن طريق تقييم نوع الأجسام المضادة IgG و IgM،حيث تم تسجيل نسبة 51 (83.61 ٪) و 10 (16.39) للعدوى الحادة والمزمنة من البروسيلا على التوالي. أظهرت النتائج التي توصل إليها اختبار iELISA الغير المباشر نسبة 42 (68.8 ٪) كانت إيجابية في حين وجدت نسبة 19 (13.15 ٪) سلبية. وبين البحث أن 18 (29.50 ٪) من الكباش وجود اعراض السريرية التي تمثلت بالتهاب البربخ وارتفاع درجة الحرارة والتهاب الخصيتين. كشف التحليل الاحصائي عدم وجود فروق فروق معنويه بدلالة (P-value>0.05) بين اختبار iELISA و ME2. استنتجت من هذه الدراسة أن مرض البروسيلا مستوطن في محافظة السليمانية. بالإضافة إلى انتشار المرض في قطعان الأغنام عن طريق الاتصال الجنسي . أخيرًا ، يجب تنفيذ برنامج عالمي يتمثل في التطعيم وكذلك السيطرة على نقل الحيوانات واستبعاد الكباش المصاب من حقول الاغنام.

الكلمات المفتاحية: اليزا الغير المباشر، اختيبار روزبنكال، 2ميركابتوايثانول،البروسلا،الكباش