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vetmed@uoKufa.edu. iq

Serological Investigation Of Caprine Brucellosis At Saniyah District

Mohsen Alrodhan

* Depart. Of clinical laboratory sciences, collage of pharmacy, Al- Qadisiya university,
Email : moh.alrodhan@gmail.com

Abstract

Brucellosis is important zoonotic disease in Iraq affect variety species of animals, cattle, sheep goats, camels and horses and transmitted to human being by direct contact with diseased animals or by ingestion of contaminated unpasteurized milk and milk products .The disease characterized by chronic nature and the infection may develop unobserved which lead to local clustering of infected cases. Serological survey was conducted to detect prevalence of brucella antibodies among goats by random selection of 120 animals in two villages at Saniyah out of target population of 848 in 32 villages. Rose Bengal test used for detection of IgM antibodies by using standard Brucella antigen (Synbiotics,Lyon, France), indirect ELISA kit (NovaLisa, Germany) was applied to investigate the seroprevalence of brucellosis in goats of studied area, the results of collected serum samples which subjected to RBT & i ELISA were 10.8 % and 12.5 % respectively,the positive results of i ELISA were higher than that of RBT which was significantly differed .The seropositivty in male 20% were higher than female 12.17% with significant difference , while the seroprevalence in relation to age groups were reported in adult animals only 14.4% and 16.6 by using RBT and ELISA respectively and there were no positive results in young ones .

In conclusion brucellosis is still spread among goats and host risk factor comprise age and sex in addition to the types of serological test influence the seroprevalence percentage, more attention should be directed towards preventing of caprine brucellosis by application of proper control programs.

Keywords (Brucellosis, goats, RBT, i ELISA, Saniyah).

الاستقصاء المصلي عن مرض البروسيلا في الماعز في ناحية السنية

محسن الروضان

فرع العلوم السريرية و المختبرية, كلية الصيدلة, جامعة القادسية

الخلاصة

يعد مرض البروسيلا من بين أهم الامراض المشتركة في العراق, يصيب عدة انواع من الحيوانات كالماشية والماعز والاعنام والجمال و الخيول وينتقل الى الأتسان عن طريق التماس المباشر مع الحيوانات المصابة او باستهلاك الحليب غير المبستر ومنتجاته. تم إجراء التحري المصلي عن أزداد البروسيلا في الماعز عن طريق الانتخاب

العشوائي ل 120 حيوان في قريتين من قرى ناحية السنية من مجموع العدد الكلي المستهدف 848 في 32 قرية, وقد تم التحري عن الاضداد IgM بواسطة فحص الـ روز بنكال (RBT) باستخدام المستضد القياسي للبروسيلات والمستورد من شركة سينبايوتك الفرنسية كذلك فقد تم الكشف المصلي عن انتشار المرض باستخدام عدة تشخيص الـ اليزا غير المباشر (i ELISA) للشركة الالمانية نوبا (Novalisa). كانت نتائج فحص عينات المصل باستخدام فحص الـ روز بنكال و الـ اليزا الغير المباشر 10.8% و 12.5% على التوالي بارتفاع معنوي لفحص الـ اليزا في نسب الانتشار, كما بينت نتائج الدراسة ان الامصال الموجبة في الذكور بلغت 20% وهي أعلى منها في الاناث 12.17% مع وجود فرق معنوي, بينما سجلت الحيوانات البالغة فقط النتائج الموجبة 14.4% و 16.6% في فحصي الـ روزبنكال و الـ اليزا على التوالي في حين لم تسجل الاصابة في الحيوانات صغيرة العمر.

يمكن الاستنتاج ان مرض البروسيلات لازال منتشر بين الماعز وان عوامل الخطورة كالعمر والجنس بالإضافة الى نوع الفحص المصلي المستخدم قد يؤثر في نتائج نسب الانتشار وبناءً على ذلك يجب توجيه الاهتمام لمنع انتشار المرض في الماعز عن طريق تطبيق برامج سيطرة مناسبة.

الكلمات المفتاحية : داء البروسيلات , فحص الـ روز بنكال, فحص الـ اليزا , الماعز , السنية

Introduction

Br. Melitensis is highly pathogenic and the infection in goats create a great risk for people and animals furthermore the disease in goats is more severe and prolonged than that in sheep (18,19).

Diagnosis of the disease can be performed by bacterial culture from aborted fetal stomach contents or fetal fluids also vaginal discharge , placenta and milk may used for this purpose , but the isolation of brucella from infected animals not always possible and practical, therefore bacterial culturing often negative (20,21,22) . So it is essential to use serological examinations for detection of specific antibodies which raises against brucella antigen during and after exposure (23,24) .

The aim of present study is to evaluate the seroprevalence of caprine brucellosis in Saniyah district by RBT & i ELISA.

Materials and methods

A total of 120 blood samples of different sex and age goats with history of abortion and known to be not vaccinated against brucellosis were collected by random selection & probability proportional sampling in two villages of Al-Saniyah district/

Caprine brucellosis is the most important zoonotic disease caused by *Brucella melitensis* causes abortion and significant losses from decrease of production (1), *Br. melitensis* is considered to reemerging pathogen in middle east(2,3). Abortion is the major clinical feature of the disease which is usually occurs at late stage of gestation, although many other clinical forms may develop such as retention of placenta , arthritis and hygroma, brucellosis may cause also metritis and subclinical mastitis in females animals and orchitis in males. (4). In human the disease is serious debilitating and sometimes chronic and can affect many organs (5). Most of cases results from occupational exposure to infected animals or ingesting contaminated diary unpasteurized products of different species of food producing animals (6,7,8,9,10).

The incidence of brucellosis in human in middle east countries may reach 78 cases per 100.000 the disease is widely spread in developing of low and middle income countries. (11,12,13,14), In Iraq brucellosis is serious endemic among susceptible animals and has public health threat (15,16 ,17).

The data was entered in Excel (Microsoft) and analyzed with descriptive on different groups by using chi square (χ^2) test to determine the significance of difference of seroprevalence of caprine brucellosis.

Results

The results of total number of 120 goats serum samples which examined for evidence of brucellosis by RBT and iELISA test, were 10.8 % and 12.5 % respectively, indirect ELISA test reported higher seropositivity than RBT with significant differences at $p < 0.05$ (Table-1)

In relation to the sex groups all bucks (goat males) were seronegative by using RBT while does (goat females) seroprevalence percentage were 12.5%, one of five bucks were seropositive by using indirect ELISA test with seroprevalence percent of 20% as compare with seroprevalence of does 12.17 % with significant difference at $p < 0.05$ (Table-2).

The result of the study in relation to the age groups Y (young) up to 11 months and O (old) ones above 11 months, showed that young kids not reported seropositive result by using RTB and iELISA while the seroprevalence percent of old goats were 16.6 % (Table-3)

Diwanayah out of target population of 848 in 32 villages, according to (24), five (5 ml) of blood were aseptically collected in sterile venipuncture and transported to laboratory in ice box as soon as possible, serum was separated within 12 hours of collection and stored at 20° c until further examination.

The collected sera were screened by RBT for the presence of IgM antibodies by using standard brucella antigen (synbiotices, France), 25 ml of RBT antigen was added on glass slide, next to 75 ml of collected goats serum and mixed thoroughly and shaken for 4 minute then agglutination was observed (18).

iELISA was performed according to manufactures instruction Kit-NovaLISA, the reagents of the kit and serum samples were brought to room temperature, serum samples were diluted. Samples, positive and negative control were transformed into wells of microtiter plate. The plate contents were mixed followed by incubation at 37° C for 1 hour. Then contents were discarded and the plate was washed. Hundred (100) ml of diluted conjugate was added to each well another incubation and washing cycle has been apply after that the substrate was added to the wells and incubation for 15 min at room temperature, then the reaction was stopped by addition of sulphuric acid to the wells. and absorbance was read at 450 nm and the OD value were used to determine the positive results.

Table (1) Seroprevalence of brucellosis in regard to types of diagnostic tests of studied goats.

Test	No.of tested animal	Positive	Percentage %
RBT	120	13	10.8
i ELISA	120	15	12.5

Table (2) Seroprevalence of brucellosis in regard to sex groups of studied goats.

Test	Sex	No. of test	Positive	Percentage %
RBT	Male	5	0	0
	Female	115	15	12.5
ELISA	Male	5	1	20
	Female	115	12	12.17

Table (3) seroprevalence of brucellosis in regard to age groups of studied goats.

Test	Age group	No. of test	Positive	Percentage %
RBT	Y	30	0	0
	O	90	13	14.4
ELISA	Y	30	0	0
	O	90	15	16.6

Discussion

Brucellosis still remains one of major bacterial problems in Iraq that affect animal production as well as human health (24, 25). The genus *Brucella* cause infection responsible for important economic losses by abortions, clinical forms of disease and reduction of milk yield in addition to the trade sanctions by constrains free animal movement (24).

A propitiate diagnosis of the disease can be performed by rapid, reliable specific and sensitive test procedure, RBT & i ELISA are most commonly used for this purpose. In the current study the seropravrance of caprine brucellosis was determined to be 10.8 by RBT and 12.5 % by i ELISA in goat flocks with history of abortion.

Application of two serological testing procedure with high sensitivity by first screening the goat flocks by RBT and after that using i ELISA as confirmative test to improve the efficacy of detecting *Brucella* antibodies. RBT as screening test is technically easy and cheap which explain the more frequent use as a routine test in Iraq. ELISA consider more specific and sensitive than RBT, ELISA as confirmative diagnostic test has possibility to differentiate between infected and vaccinated animal (23).

Diagnosis is often based on serological methods as bacterial examination and culturing is not practicable for routine application (24). In other study conducted on sheep at Basra by (27), the positive results by

using RBT & indirect ELISA were 54.4 % & 68.8 %, respectively, the relative low seroprevalence of caprine brucellosis in this study may be due to grazing conditions that reduce contact among animals in addition to decrease contamination of pasture under dry climatic environment, another reason could be owners of studied area don't introduce animals from the market and depend on their own flocks. Also the average of flocks size of goats generally smaller than sheep with intensive population.

The results in relation to sex showed that there were no positive results has been reported in males by using RBT, while in females were 12.5% on the other hand the results of i ELISA showed that the seroprevalence in males were 20% as compare with 12.17% in females with significant difference at $p < 0.05$.

The high seropositivity in male may be due to long period of exposure of little number of bucks used in natural service of the chance of infection may increase during the sexual intercross from infected female, the result not agree with the finding of (27), male and female goats are equally susceptible to the disease however significant difference in the seroprevalence between them has been reported also the average of flocks size of goats generally smaller than sheep with intensive population.

A higher seroprevalence of brucellosis were stated in female by other study (26). The results of present study indicate that sex influence the rate of infection however the facts responding in the incidence of brucellosis in relation to sex is controversial (28) found that females were higher prevalence than males.

The seroprevalence of caprine brucellosis according to age groups were showed no positive results has been reported in young animals by using both serological test while old goats seropositivity results were 14.4% and 16.4 % by using RBT and ELISA respectively.

High seroprevalence in old goats (above 11 months) and entire negativity of young animals up to 11 months may be due to the lack of erythritol which present in mature genital organ and gravid uterus.

Erythritol (sugar alcohol) play important role in the pathogenesis of brucellosis by stimulation of bacterial growth. The result of this study revealed that the seroprevalence of caprine brucellosis in study area is still important and the type of serological tests, sex and age influence the results of seroprevalence. These findings ensure the need for application of effective control measures & disease prevention which increase health awareness on transmission method and human infection.

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