Significant of conventional serological tests in diagnostic of Brucellosis in AL-Diwaniyah province

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الخلاصة

شملت الدراسة 351 عينات مصول من الحالات المشكوك بإصابتها بمرض البروسيلوسز ، في الانسان تم جمع 125 عينة (68 ذكور و 57 اناث) و بأعمار تراوحت بين 10 – 70 سنة و 100 عينة من الاغنام (16 ذكور و 84 اناث) ، الماعز (17 ذكور و 23 اناث) و الابقار (22 ذكور و 64 اناث) و بأعمار تراوحت بين اقل من سنة الى اكثر من ثلاث سنوات ، في الفترة من تشرين الاول 2011 الى اب 2012 في محافظة الديوانية . استخدمت اختبارات وردية البنكال و اختبار التلازن الانبوبي و اختبار 2-ميركابتوايثانول لمعرفة حدوث داء البروسيلا . سجلت نسبة عالية للمرض باستخدام اختبار وردية البنكال و كانت 19,2% و 21% و 17,39% و 8,13% في الانسان والاغنام و الماعز والابقار على التوالي ، كانت النسبة العالية للإصابة في الانسان عند الذكور بينما في حيوانات الماشية كانت اغلب الاصابات في الاناث في جميع الفحوصات المصلية عند العمر 1-3 سنة ، بينما في الذكور كانت عند عمر اكثر من 3 سنوات . نتائج اختبار التلازن الانبوبي تشير الى ان المعيار320 / 1 كان اكثر تكرارا" مقارنة" مع المعايير الاخرى . كانت الحالات المزمنة باستخدام اختبار 2-ميركابتوايثانول ثمان حالات في الانسان و 4 و 1 و 2 في الاغنام و الماعز و الابقار على التوالي . كانت نسبة توافق العينات السالبة لاختبار وردية البنكال و اختبارات

الانبوبي و 2-ميركابتايثانول 99% و 99% في الانسان على التولي و 98,43% و 98,43% في الاغنام فضلا"عن 100% للأبقار و الاغنام.

Abstract

The study was conducted on 351 serum samples from suspected cases brucellosis of human (125) (68 male and 57 female)of age distribution from (10-70) years and 100 sheep (84 females and 16 males) , goat (23 female and 17 male) and cow (64 female and 22 male) of different ages (1->3 years) suspected selected from October 2011 to August 2012 in Diwaniyah province. The Rose Bengal test, tube agglutination test and 2- mercaptoethanol test were used to determine the incidence of brucellosis. The highest infection rate of disease was recorded by Rose Bengal test 19.2%, 21% , 17.39% and 8.13% in human sheep ,goat and cow respectively . In human the high incidence was in male where as in livestock animal the highest incidence was in females in all serological tests and the highest incidence was in females at the age between 1-3 years whereas in males more than 3 years of age .The results of tube agglutination test revealed the titer 1/320 occurred mostly compared with other titers. Eight chronic cases were determined by 2-mercapto-ethanol test in human and 4 ,1 and 2 chronic cases in sheep , goat and cow respectively. The degree of agreement of negative samples with Rose Bengal test and tube agglutination, and 2-mercaptoethanol tests was 99%, 99% in human and 98.43% and 98.43% in sheep, as well as 100% in both test for goat and cow respectively .

Introduction

Brucellosis is a bacterial zoonosis of worldwide importance, and of major public health and economic significance (1,2).The causing agents are Gram-negative, facultative intracellular coccobacilli or short rods from the family Brucellaceae that localize in the reproductive organs of host animals, causing abortions, fetal death, genital infections (3) The disease is endemic in many countries especially around Latin America, Mediterranean and Middle East

countries (2, 4). Animal infections most commonly occur through contact with infected fetal tissues and post-parturient discharges. Human infections occur from contact with infected animal tissues or ingestion of infected animal products (5). The diagnosis of brucellosis can be based on cultural isolation, serological tests and in recent year by biotechnological techniques. Cultural isolation is time consuming, cumbersome and requires specialized laboratory personnel. Molecular based detection is rapid and sensitive require establishment of advanced laboratories and trained personnel. At present mainly serological methods are used for diagnosis of Brucellosis infection in India. The long-term serological studies at national level have indicated that 5% of cattle and 3% of buffaloes could be infected with brucellosis (6). After reviewing various serological tests no individual test found perfect, however, error could be minimized using the most reliable test (7)

The serological methods are usually employed for diagnostics of *Brucella* in blood specimens. The serological response, however, can be unspecific due to cross-reaction or sub sensitive reactions in samples from areas with a low or subclinical prevalence of brucellosis (8). These techniques could be potentially useful for the diagnosis of brucellosis since they could detect the bacteria in paucibacillary samples and even in samples highly contaminated with other microorganisms (9).Consequently, detection and identification of *Brucella* spp. in clinical specimens by cultures may still be a difficult task with significant delays, For these reasons serology has been the mainstay of diagnosis of Brucellosis(10) . According to the above information the aim of the study was to evaluate the use of convention serological technique for rapid and specific diagnosis of Brucellosis obtain from serum samples of human and livestock animals .

Material and Methods

In human 125 serum samples has been collected (68males and 57 females) these ages were range from 10-60 years ,The samples collection depending of clinical signs of suspected cases were recorded by body temperature, sweating, bleeding , headache, arthritis and abdominal and back pain ….. etc. Three to Five ml of blood samples collected from each person, the serum has been stored by freezing (-20 Cº) until used for serological tests. In livestock animal, a total of 226 blood samples were cows(86) and from sheep and goat (140) were collected their ages were range from < 1 years to > 3 years. 5-10 ml of blood was withdrawn from each animal the serum has been stored by freezing (-20 Cº) until used for serological and molecular tests .

The RBPT antigen and B. abortus plain antigen for STAT were used. The RBPT and STAT were performed as described by (11). Definite clumping/agglutination was considered as positive reaction, whereas no clumping/ agglutination was considered as negative for RBPT while serum titer of 80 IU or above were considered to be positive, 40 IU as doubtful and less than 40 IU as negative for STAT (11). the Mercaptoethanol solution , this solution prepared dissolved 1.4 ml of 2-mercaptoethanol and add to complete 1000 ml from sterile physiological saline 0.85% that it's use during one week from preparation (11) which it's used in dilution of serum and antigen in 2-mercaptoethanol test (2-ME) .

Results

Among the 125 cases screened, 24 cases were positive by Brucella Slide Agglutination test male (26.47%) than female (10.52%) table (1) . In sheep from 100 sample of abortion herd and suspected case 21 cases (21%) were positive by rose Bengal slid test , 18 /84(21.42%) related to female which more than males 3/16(18.75%). as well as, in goat 6/40 (15%) from abortion and suspected cases of goat serum sample. cases, male 2/17(11.76) as well as the female 4/23 (17.39%) and in cattle 7/86(8.13%) cases were positive , the female gave 5/64 (7.81%) while in male 2/22(9.09%) table (2) . In human the distribution of brucellosis infection due to Rose Bengal test according age groups showed the infection rate from (21-40) age in males and females also total of percentage in these groups more than others(table 3) , The majority of infected animal in sheep, whose serum samples were received for some febrile agglutination test, belong to the age group of more than three years as well as in the goat ,also the major sample of infected cow belong to age at more than 3 years (table 4)

Table 1 : occurrence of brucellosis by slid agglutination test according to sex in human

Animals	Sex	Slid agglutination test		Total
		Positive	Negative	
Human	Male	18 (26.47%)	50 (73.52%)	68
	Female	6 (10.52%)	51 (89.47%)	57
Total		24 (19.2%)	101 (80.8%)	125

Table 2 : occurrence of brucellosis by slid agglutination test according to sex in livestock animals

Animals	Sex	Slid agglutination test		Total
		Positive	Negative	
Sheep	Male	3 (18.75%)	13(81.25%)	16
	Female	18 (21.42%)	66(78.57%)	84
Total		21 (21%)	79(79%)	100
Goat	Male	2(11.76%)	15(88.23%)	17
	Female	4(17.39%)	19(82.6%)	23
Total		6(15%)	34(85%)	40
Cow	Male	2(9.09%)	20(90.9%)	22
	Female	5(7.81)	59(92.18%)	64
Total		7(8.13%)	79(91.86%)	86

Table 3 : Association of age and the infection of Brucellosis in human by Slid agglutination test

	10-20	21-30	31-40	41-50	51-60	61-70	Total
Infected	3(12.5%)	8(33.3%)	8(33.3%)	3(12.5%)	1(4.1%)	1(4.1%)	24
Non infected	18(17.8%)	37(36.6%)	18(17.8%)	11(10.8%)	11(10.8%)	6(5.9%)	101
Total	21(16.8%)	45(36%)	26(20.8%)	14(11.2%)	12(9.6%)	7(5.6%)	125

Table 4 : Association of age and the infection of brucellosis in livestock animals by Slid agglutination test

Species	No. of positive cases according age distribution			Total
	< 1 years	1-3 years	>3 years	
Sheep	2(9.5%)	7(33.3%)	12(57.14%)	21
Goat	0	3(50%)	3(50%)	6
Cow	0	2(28.57%)	5(71.4%)	7

A scarified antibody titer in STAT showed 1/320 is the most frequencies in human and sheep also 1/640 showed in livestock animals(table 5) , as well as used 2-ME for distinguish between acute and chronic cases that appeared 8 , 4, 1,and 2 chronic cases in human ,sheep goat and cow respectively (table 6)

Table 5: SAT results in human , sheep ,goat and cow

species	+ve in rose Bengal	+ve SAT	Agreement percentage	Titer in IU						
				10*	20*	40	80	160	320	640
Human	24	22	91.66 %	1	1	2	5	5	7	3
Sheep	21	19	90.47 %	1	1	2	3	3	5	6
Goat	6	5	83.33	1	_	1	_	1	2	1
Cow	7	5	71.42	_	2	1	1	_	1	2

Key :(*) negative value

Table 6: No. of acute and chronic samples according to 2-ME test in human and livestock animals and compared with RBPT and SAT

Species	No of examined Samples	2-ME test	
		Acute infection Titer <40 IU	Chronic infection Titer > 40 IU
Human	125	14	8
Sheep	100	15	4
Goat	40	5	1
Cow	86	5	2

Discussion

Brucellosis is a zoonosis transmittable to humans, it's shows a high degree of morbidity, both for animals and humans. Consequently, it causes significant financial loss and represents a serious public health problem in many countries .this study showed there is association between sex and age as well as the is variance between sex infectious ,in human the Results revealed that 50% of the patients who diagnosed as having brucellosis belong to the age group of 20-40 years. also the males and females ratio of patients suffering from brucellosis is 3:1. This finding were similar with (12) who reported 66.66% males and 33.33% females and found male to female ratio of 1.99:1.This is ascribed to the fact that people of this age group are the people involved in agriculture, and related work and exposed to the livestock animals hence have increased risk of contracting the disease. Similar findings have been reported by (13) who reported that 43% of the cases in their study belong to the same age group. As a results of the study showed that there is a difference infected rates depending on the age of the animal, where it was observed that the age group of one year to three years . (14) Where he stated that the incidence is high in adult animals sexually compared with small animals age ,In livestock animals ,females numbers are more than males , a review of the results of this study noted that the incidence of females is higher than the males and may be due to the increase erythritole in the womb of

pregnant females compared with the levels in the members' the male reproductive system , makes it a factor predisposing for breeding bacteria causing the disease more in females than in males , is agreement with (14 ; 15 ; 16)

Our results of Rose Bengal test showed (19.2%) of suspected cases of human in agreement with previous Another study conducted by (12) examined 618 serum samples from veterinary Personnel and found 15.69% tested positive. RBPT in sheep appeared (21%) were positive by rose Bengal is agreement with (17) showed 23.3 were positive with rose Bengal test

The Rose Bengal plat test (RBPT) in cow were positive is (8.13%) in rose Bengal test which disagree with AL-Rodhan (15) showed (33.3%) of infected cow in diwanya city and (18) observed infected rat 18.18% and (19)showed infected rat 15.7 %

The reasons attributed this difference above in the incidence of a number of samples taken and to differences in the methods of education and good governance, which vary from one field to another and from one other province as well as all the scientific background, and may be caused by increased incidence between this study and the studies referred to the non-application software control scientific and integrated disease as well as "ignorance of educators seriousness of the disease and its rapid spread and not to follow healthy ways to get rid of aborted fetuses and other pollutants. Comparing the serum agglutination test(SAT) results with a Rose Bengal test founded that the rate of its compatibility with Rose Bengal in human ,sheep ,goat and cattle were 91.66% , 90.47%, 83.33 % , 71.42% respectively ., has been observed that IgM up to a higher rate and for longer periods in infected Brucellosis and this also explains the difference in the results, as the test and RBPT reveals disease before serum agglutenation test (SAT) and the latter gives negative results at the beginning of infection as well as in chronic cases and SAT measures globulins immune IgM, IgG1, IgG2 and IgA (20), Comparing the 2-mercaptoethanol test (2-ME) results with a Rose Bengal test, the latter is unsuccessful to discover all infected cases that gave positive with RBPT .In human compatibility with Rose Bengal is (33.33%), while in sheep, goat and cow are 19.4%, 16.66% and 28.57% respectively. There is different between 2-ME and RBPT results because 2-ME breaks the disulphide links of IgM pentamer, thus interfering with its highly efficient agglutinating capacity while not affecting IgG molecules(21) . The reason for this difference is due to differences in the efficiency of these tests to detect antibodies in various stages of the disease and that the 2 - mercaptoethanol used to detect infected chronic in animals, making it does not give positive results in infected animals extremes sharp (22) as well as sometimes it be clumping in these tests (Rose Bengal , SAT and 2-ME) is a qualitative Non-specific because there cross-reaction between *Brucella* and other germs leading to stimulate antibodies appear false-positive result (23) .

Conclusion and recommendation

Serological test gave rapid screening test for detection of brucellosis . The possibility of using the serological test as a Rapid examination of infected Human and livestock animals by using (RBPT , STAT and 2-ME) as screening method for diagnosis of brucellosis and differentiation between acute and chronic brucellosis .

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