LABORATORY DIAGNOSIS OF BRUCELLOSIS USING THE SLIDE AND STANDARD TUBE AGGLUTINATION METHODS*

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ملخـــص

في هذا البحث قمنا بفحص ١١٢ عينة دم لتشخيص مرض الحمى المالطية بطريقة Rapid slide and standard tube agglutination method وقد وجدنا ان ٥٦ ٪ من المرضى عندهم titer أقل من 20:1 بينما 33 % عندهم titer أعلى من 80:1 .

وقد وجدنا ان هناك تناسبا في استعمال الطريقتين حيث كانت نتائج كلا الطريقتين مشابهة لنتائج الطريقة الاخرى .

Abstract

Both the rapid slide and standard tube agglutination methods were used for the diagnosis of 112 blood specimens for Brucellosis . We found that 56% of the patients have a titer < 1:20 while 33% have a titer > 1:80 .

We found that the rapid slide agglutination method correlates well with the tube agglutination method .

Introduction

Brucellosis is an epidemic disease in the West Bank of Jordan . It is a zoonotic disease in which infection is transmitted to humans from domestic animals through direct contact or mostly through consumption of unboiled milk and milk products taken from infected animals .

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In the West Bank , people most commonly get the infection from eating raw white cheese prepared from the milk of infected goats , sheep or cows . The people who are involved in cheese production , do not boil or pasteurize milk before using it The disease starts to appear early in Spring and continues through Summer . This is due to the fact that white cheese appears in the market in Spring .

Several serological tests are used for the diagnosis of the disease [1,2,6,7] . The agglutination method is one of these methods . It is easy to do in private small laboratories . We undertook this study to determine whether to use the rapid slide agglutination method or the standard tube agglutination method .

Experimental

A total of 112 blood specimens withdrawn from patients showing the symptoms of Brucellosis . Sera of the patients blood were separated after clot formation . **Brucella abortus** antigen (Gamma diagnostics) , 0.85% saline , positive and negative control sera , serological pipettes , test tubes 13 x 100 mm and slides divided into 12 squares were used in the study .

Both the slide and tube methods were carried out according to the instructions of the manufacturer [3] .

Rapid Slide Method

Using a micropipette , the following serum volumes were delivered to the already divided slide : 0.08ml, 0.04ml, 0.02ml , 0.01ml and 0.005 ml . These serum volumes are approximately equivalent respectively to the following titers 1:20 , 1:40 , 1:80 , 1:160 or 1:320 . Then one drop of **B. abortus** antigen after shaking the vial was added to each dilution . The dropper of the vial was used to deliver the drop of antigen which is equal to 0.05ml. Using an applicator stick , the serum and antigen in each square were mixed and spread . The slide was rocked gently back and forth for no longer than 3 minutes . After that agglutination was observed both macroscopically and microscopically . The highest dilution which showed agglutination was considered the titer .

Both negative and positive control sera were treated as mentioned above.

Standard Tube Method

A series of 10 (13 x 100mm) test tubes were placed in a rack . Then 0.9ml saline was delivered in the first test tube and 0.5ml in each of the remaining test tubes . After

that 0.1ml of the tested serum was added to the first test tube . After mixing , 0.5ml of the diluted serum was transfered to the second test tube . Then 0.5ml diluted serum was transfered from the second test tube to the third test tube , and so on until the contents of tube 10 were mixed , from which 0.5ml diluted serum was discarded . The resulting dilutions in the 10 test tubes ranged from 1:10 in tube no. 1 to 1:5120 in tube no 10 . As an antigen control another tube was added to the series containing 0.5ml saline . Then 0.5ml **B. abortus** antigen diluted 1:50 in saline , was added to each tube to make a final dilution varying from 1:20 to 1:10240 . After shaking the rack well , it was placed in a 37 °C water bath for 48 hours . The same procedure was repeated at the same time for positive and negative controls .

Results and Discussion

Up to date the agglutination method is used by most laboratories for the diagnosis of brucellosis . However , reports dealing with the usefulness of ELISA for diagnosing human brucellosis have described highly satisfactory results [1,6] . Still the bacteriological examination is the most sensitive method [7] .

In our study as shown in table 1,63(56%) of the patients have a titer of $< 1:20$ using the slide and tube methods . While 9 (8%) of the patient have 1:20 titer using the slide method and 1:40 using the tube method . Two patients (1.8%) have 1:40 titer using the slide method and 1:80 using the tube method . One patient (0.89%) has 1:80 titer in both methods . 2(1.8%) patients have 1:80 titer using slide method and 1:160 titer using tube method . Five patients (4.5%) have 1:320 titers using both slide and tube methods . One patient (0.89%) has 1:160 in slide method and 1:320 using tube method . Twenty eight (25.2%) patients have a titer $> 1:320$ using slide method and titers ranging from 1:640 to 1:5120 using tube-method . Patients having titers above 1:80 or showing a raising titer are considered to be infected with brucellosis [4] .

Our results show that the slide method correlates well with the tube method . This is in accordance with other studies [5] . Both methods have almost the same results with slight differences in the titer in few cases . Thus we suggest to use the rapid slide method as a screening test and to use the tube method to establish the titer in doubtful cases . We found that the tube method may give high false positive results in unexperienced hands due to false positive agglutination . In addition to this , glass should be completely clean and free of any contaminants .

Our recommendation is to do the rapid slide method , but doing the tube method just to find out the actual titer in some doubtful cases only .

Serum titers equal to 1:80 using the slide method should be preferably repeated using the standard tube method , as this is a borderline titer .

Table 1 : A comparison between rapid slide and tube agglutination methods .

Number of Serum Specimens	Rapid Slide Method titer	Standard Tube Method titer	%
63	< 1:20	< 1:20	56.00
9	1:20	1:40	8.00
2	1:40	1:80	1.80
1	1:80	1:80	0.89
2	1:80	1:160	1.80
1	1:160	1:160	0.89
5	1:320	1:320	4.50
1	1:160	1:320	0.89
13	>1:320	1:640	11.60
7	1:320	1:1280	6.30
5	1:320	1:2560	4.50
3	1:320	1:5120	2.70
Total 112			99.87

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