

## **DETERMINATION OF IDEAL TIME OF *BRUCELLA* DIAGNOSTIC METHODS IN ABORTION COWS**

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### **ABSTRACT**

The aim of this study was to determine of ideal time of Brucella diagnostic methods in abortion cows. For this purpose, fifty six suspected abortion cow by Brucella were studied by brucella culture method, rose Bengal test, tube agglutination test, Mercaptoethanol test and ELISA test. These tests applied during first week, third weeks and sixth weeks of abortion.

The study results revealed the first week of abortion was the ideal time for apply of bacterial culture and tube agglutination test with positive ratio (32.1%). while the ideal time of rose Bengal test at third week of abortion in positive ratio (32.1%).

The ideal time of Mercaptoethanol test at sixth weeks of abortion with positive ratio (28.5%), while the ideal time of iELISA test at third weeks or sixth weeks of abortion with positive ratio (32.1%).

### **INTRODUCTION**

Brucellosis is a zoonotic bacterial disease and highly distributed which causes abortion in pregnancy animals and infertility in males(1). Brucella usually transmitted between animal and to humans by contact with the placenta, fetus, fetal fluids and vaginal discharges from infected animals,(2). Brucella are Gram-negative coccobacilli or short rods, they arranged singly and less frequently in pairs or small groups, non-motile, not spore forming, Stained by modification of Ziehl-Neelsen method (3).

Brucellosis can be diagnosed by two main methods. First method is called direct method which can be made by smears staining with Stamp's method, bacterial culturing and PCR. Second method is called indirect method which include rose Bengal test, plate agglutination test, complement fixation test, tube agglutination test, mercaptoethanol test, ELISA, gel precipitation test, immuneocapture test, fluorescence polarisation assay and Coombs test, other types of indirect test include brucillin test and macrophage migration test which detect of cellular immune response (4,5).

## MATERIALS AND METHODS

- 1- Samples:
  - a- Vaginal swab collected in periods ( 56 swabs within first week, 56 swabs in third week and 56 swabs in sixth week of abortion from same animals )then kept in Trypton soya broth (Himedia- India) until bacterial culturing in selective media.
  - b- Serum samples were collected in periods( 56 serum samples within first week, 56 serum samples in third week and 56 serum samples in sixth week of abortion from same animals ) and stored in freeze until serological test application.
- 2- Bacterial culture: a bacterial culture has been applied according to Alton et al., 1975 (6) by using brucella basal media (Biolife- Italy) with selective supplement (Himedia- India) and add aseptic horses serum in percentage 5%. Each sample cultured by use two petri dishes, one incubated aerobically and other incubated in 2.5-5% CO<sub>2</sub> by used gas bag (Himedia- India) for 7days when brucella appeared, sub culturing and biochemical test were applied according to Quinn et al., 2002 (7).
- 3- Serological tests
  - a- Rose Bengal test: used a kit from (Spinreact- Spanish) the agglutination within 4 mints consider a positive result.
  - b- Tube agglutination test: used a kit from (Morganville-USA). The titer 40 consider a positive result (3).
  - c- Mercaptoethanol test: used a kit from (Morganville- USA) and serum dilution by 2- Mercaptoethanol stain. The titer 40 consider a positive result (3).
  - d- iELISA test: used kit from (Savnova- Spanish) the Optical density measured under 450 nanometers and the positive result must be equal or more than 15.

## RESULTS

The brucella isolate give a typical growth characters, colony morphology, bacterial stains and biochemical characteristics that descript by Quinn et al., 2002 (7).

Within first week of abortion: the positive ratio of bacterial isolation, rose Bengal test, tube agglutination test, Mercaptoethanol test and iELISA are: 32.1%, 12.5%, 32.1%, 3.5%, 8.9% respectively. (Table 1)

Third week of abortion: the positive ratio of bacterial isolation, rose Bengal test, tube agglutination test, Mercaptoethanol test and iELISA are: 19.6%, 32.1%, 25.0%, 16.0%, 32.1% respectively. (Table 1)

Sixth week of abortion: the positive ratio of bacterial isolation, rose Bengal test, tube agglutination test, Mercaptoethanol test and iELISA are: 3.5%, 28.5%, 8.9%, 28.5%, 32.1% respectively. (Table 1)

**Table (1): Positive result of bacterial culture and serological test used in present study**

Period after abortion	Positive results of tests									
	Brucella isolation		Rose Bengal test		tube agglutination test		Mercaptoethanol test		iELISA test	
	No	%	No	%	No	%	No	%	No	%
within first week	18	32.1%	7	12.5%	18	32.1%	2	3.5%	5	8.9%
after three weeks	11	19.6%	18	32.1%	14	25.0%	9	16.0%	18	32.1%
after six weeks	2	3.5%	16	28.5%	5	8.9%	16	28.5%	18	32.1%

## DISCUSSION

There are many bacterial infectious causes of cow abortion, but the most important was brucellosis which occur in the terminal stage of pregnancy (8,9). The gold standard for diagnosis of brucellosis is a bacterial culture (4).

The present study showed that the ideal time for using of bacterial culture was within first week of abortion. This result agreement with result showed by (10) that said the bacterial culture successful in diagnosis of brucellosis in ratio reach to 90% in early stage of infection.

In most infection state the immune response occur in two stage, in first one produce of IgM, which detected by use tube agglutination test (3, 5,11). The present study indicated time for tube agglutination test was within the first week of abortion.

The present study showed that the ideal time for rose Bengal test was on third week of abortion. This may be due to of that the abortion is evidence of early infection (12), and this test detect two types of immunoglobulins (IgM, IgG) but its more efficient of immunoglobulins IgG(6).

**Nielsen** (2002) stated that IgM appear within 1-2 weeks after infection with low level of IgG, and then the titer of IgG began to rise while IgM began to decline (13). The present study showed that the ideal time for Mercaptoethanol test at sixth week of the infection. It's due to ability of Mercaptoethanol to smash IgM and leave IgG which indicate to chronic infection (14).

The present study showed that the ideal of iELISA test at third week and sixth week of abortion, which may be due to type of the kit (kit detect IgG). ELISA has ability to detect low level of immunoglobulins when compare with other types of serological tests (15).

## تحديد الوقت المثالي لإجراء بعض الاختبارات التشخيصية لداء البروسيلات في الأبقار المجهضة

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### الخلاصة

هدفت هذه الدراسة الى تحديد الوقت المثالي لاختبار الاختبارات التشخيصية لداء البروسيلات في الأبقار المجهضة ، ولهذا الغرض تم دراسة ٥٦ حالة إجهاض في الأبقار مشكوك بإصابتها بداء البروسيلات باستخدام الزرع الجرثومي، اختبار وردية البنغال ، اختبار التراص في الانابيب، اختبار المركبتوايثانول واختبار الممتز المناعي المرتبط بالإنزيم في فترات (ضمن الاسبوع الاول من الإجهاض، الاسبوع الثالث من الإجهاض، الاسبوع السادس من الإجهاض).

أظهرت نتائج الدراسة أن أفضل وقت لإجراء الزرع الجرثومي واختبار التراص في الانابيب هو ضمن الاسبوع الاول من الإجهاض وبنسبة (٣٢.١ %). وأفضل وقت لإجراء اختبار وردية البنغال هو الاسبوع الثالث من الإجهاض وبنسبة إيجابية بلغت (٣٢.١%). في حين كان الوقت المثالي لإجراء اختبار المركبتوايثانول هو الاسبوع السادس من الإجهاض وبنسبة إيجابية بلغت (٢٨.٥%). إما اختبار الممتز المناعي المرتبط بالإنزيم فأفضل وقت لإجرائه هو بعد ثلاثة اسابيع وبعد ستة اسابيع من الإجهاض وبنسبة إيجابية بلغت (٣٢.١%).

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