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COMPARISON OF SOME RAPID TECHNIQUES USED FOR DIAGNOSIS OF BRUCELLOSIS OF ABORTED COWS COMPARED WITH CONVENTIAL METHOD

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ABSTRACT

A total of 158 specimens of uterine discharges and lochia were collected from aborted cows of different localities in Egypt. The blood samples of the same aborted cows were also collected for serological tests. The bacteriological method was applied for isolation of *Brucella* organisms from 102 uterine discharges and 56 lochia of aborted cows. Primary inoculation was done on *Brucella* agar plates. The plates were incubated in the presence of 5% CO_2 in Carbon dioxide incubator for 72-120 hrs. The isolates were initially recognized on the basis of their cultural and morphological features and biochemical identification. DOT-ELISA was applied on the colonies plates for detection of *Brucella* microorganisms in the aborted materials. The direct fluorescent antibody test (DFAT) was applied on the specimens of uterine discharges and lochia. Samples were coated on the slides as antigens, then *Brucella* specific antibodies labelled with a fluorescein conjugate were added and examined under the fluorescent microscope. The applied serological tests in this study were Rose Bengal Test (RBT), Standard Tube Agglutination Test (SAT), Rivanol Test (RT) and Enzyme Linked Immunosorbant Assay (ELISA) test. The rate of isolation of *Brucella melitensis* (*B. melitensis*) from aborted cows was 7.59% from 12 isolates; 7.84% from 8 uterine discharges and 7.14% from 4 lochia by cultural bacteriological method and Dot-ELISA. The result of DFAT were 25(15.82%) positive; 16 (15.69%) from uterine discharges and 9 (16.07%) from lochia. The results of serological tests on the serum samples were 64 (40.51%), 58 (36.71%), 55 (34.81%) and 66 (41.77%) positive results for RBT, SAT, RT and ELISA respectively. Finally, we concluded that in order to eradicate and control brucellosis, we must apply a good surveillance reliable diagnostic test. The practical serological diagnosis must be based on screening test of high sensitivity followed by a confirmatory test as ELISA test of high specificity. A combination of serological test with FAT is usually needed for diagnosis of *Brucella* organisms in aborted cases.

Keywords: *Rapid techniques, Brucellosis, Cows.*

INTRODUCTION

Brucella has a significant economic impact on our livestock business. It has severe consequence on production of cattle that influences two of the greatest infertility and sterility problems, (Parker, 2003).

Brucellosis is a highly infectious bacterial disease that mainly affects cattle, sheep, pigs and goats. The organism causing brucellosis primarily infects the reproductive organs and thrives in the uterus of pregnant animals, often leading to late abortion (OIE 2001). The organism can remain undetected for prolonged periods as there are no clinical signs until abortion occurs (AHVLA, 2013).

Bovine brucellosis is the best known and most controversial infection of the bovine reproductive system. It is one of the core profiles of economic consideration in livestock production enterprises since loss of calf due to abortion and its squeal lead to infertility (Verma *et al.,* 2000).

Brucellosis infection of cattle causes abortion or premature calving of recently infected animals, the foetus, placenta and uterine fluid contain large quantities of *Brucella* organisms which can infect other animal coming into contact with an infected animal around the time of calving (Nielsen *et al.,* 2005).

The gold standard technique for diagnosis of brucellosis is isolation and identification of the causative bacterium *Brucella* species. Isolation of *Brucella* organisms requires a high secured laboratory facilities (biological containment level 3), an extended time for results, highly skilled personnel and hazardous procedure. Brucellosis is generally diagnosed by detection of antibodies in serum or other body fluids. Subsequently, various modification of agglutination test and numerous

other tests have been developed to increase test accuracy (Nielsen and Yu, 2010).

Brucellosis was firstly diagnosed by using a simple tube agglutination test by Wright and Smith (1897). The other tests have been developed to increase test sensitivity. However no test is 100% accurate. So, generally serological diagnosis consists of testing sera by several tests, usually as screening test of high sensitivity followed by a confirmatory test of high specificity (Nielsen *et al.,* 2005).

The present study was contemplated to reveal some rapid diagnostic techniques used for diagnosis of brucellosis of aborted cows as DFAT and DOT-ELISA compared with convential method

MATERIALS and METHODS

In the present study a total of 158 specimens were collected from aborted cows of different farms in Egypt (102 uterine discharges and 56 lochia). Also 158 blood samples of the same aborted cows were collected for serological tests.

The conventional bacteriological methods (Alton *et al.,* 1988) were applied for isolation and identification of *Brucella* organisms from the all specimens.

Primary inoculation was done on sheep blood agar plates in duplicate by directly streaking the swabs to be cultivated. The plates were incubated at 37°C in the presence of 5% CO_2 in Carbon dioxide incubator for 72-120 hrs. The isolates were initially recognized on the basis of their cultural and morphological features. They were also biochemically characterized as described by (Carter and Cole, 1995).

DOT-ELISA was applied on the colonies plates for detection of Brucella microorganisms in the aborted materials as described by (Nielsen *et al.,* 2004).

Direct Florescence Antibody Technique (DFAT) was applied on the specimens of uterine discharges and lochia. Samples coated on the slides as antigens and then *Brucella*_specific antibodies labeled with a fluorescein conjugate were added (Nicoletti and Tanya, 1993).

The serological tests applied on these studies were Rose Bengal tset (RBT), Standerd Tube agglutination test (SAT), Rivanol test (RT) and Enzyme linked Immunoasorbant Assay (ELISA) according to (Nielsen, 2002).

RESULTS

From 102 uterine discharge samples only 8 brucella isolates could be identified, also 4 brucella isolates could be identified from 56 lochia samples, but when we used DFAT on uterine discharge and lochia gave 25 positive brucella cases.

Serological test applied on serum obtained from 158 blood samples showed better detection of brucella antibody by ELISA 66 sample than Rose Bengal 58 positive sample.

Table 1: Brucella isolates encountered from aborted cows by culture.

Type of samples	No. of samples	Brucella isolates*	% of isolates
Uterine discharges	102	8	7.84%
Lochia	56	4	7.14%
Total	158	12	7.59%

* based on cultural, morphological and biochemical features.

Table 2: The incidence of Brucella in samples of aborted cows by DFAT and Dot ELISA.

Type of samples	No. of samples	Positive samples by DFAT	Positive samples by Dot ELISA
Uterine Discharges	102	16 (15.69%)	8 (7.84%)
Lochias	56	9 (16.07%)	4 (7.14%)
Total	158	25 (15.82%)	12 (7.59%)

Table 3: The prevalence of Brucella in serum samples of aborted cows by serological test.

Type of samples	No. of samples	Serological Tests			
		RBT	SAT	RT	ELISA
Serum	158	64 (40.51%)	58 (36.71%)	55 (34.81%)	66 (41.77%)

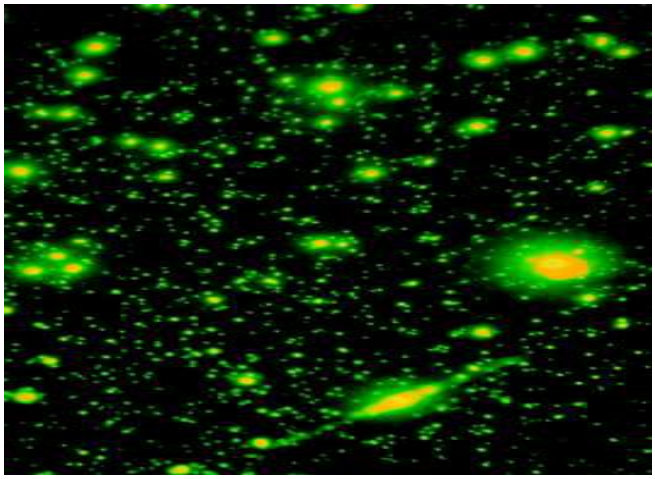

Fig.1: Positive DFAT applied on lochia of aborted cow.

DISCUSSION

Brucellosis remains a major worldwide zoontic disease (Cutler and Whatmore, 2003). It is a bacterial disaease of global importance that may affect different mammals. The disease primarily affects the reproductive system with concomitant loss in productivity of animals (Young, 1995).

Brucellosis is considered as an emerging problem in developing countries where there is an increasing incidence of *B. melitensis* in cattle (Corbel, 1997). The organisms survive within the environment for prolonged periods (Moreno and Gorvel, 2004). Interaction with placental trophoblasts suggests that the ability to acquire iron is vital as the *Brucella* enter their acute replicative stage within the placental disruption resulting in fetal loss or birth of weak and/or infected off spring (Eschenbrenner *et al.,* 2002 and Cutler *et al.,* 2005).

The accurate diagnosis of brucellosis in any species goes straight forward but may be very difficult in some cases (Nielsen and Yu, 2010). *Brucella* diagnostic tests were developed based on agglutination methods. These assays have been played with problems of both sensitivity and specificity (Alton *et al.,* 1988 and Nielsen, 2002).

Diagnosis of *brucella* infection can be made by isolation and identification of the organisms by convential methods (Bercovich, 2000). In the present study, the rate of isolation of *B.melitensis* from aborted cows was 12 isolates; 8 from uterine discharges and 4 from lochia by cultural bacteriological method and Dot- ELISA (Table, 1 & 2). These findings agree with (Zowghi and Ebadi 1988), that all the isolates of *brucella* encountered in this study were identified by biochemical tests as described by (Carter and Cole, 1995).

The obtained results revealed *Brucella* positive by direct fluorescent antibody test applied through specific binding of antibody to the provide antigen conjugated with fluorescein conjugate. The results were 25positive cases of DFAT; 16 from uterine discharges and 9 from lochias. These findings coincide with (Samartino *et al.,* 1999) and (Bahn and Nockler 2005). The higher incidence rate of *Brucella* organisms was done by DFAT which is a simple, rapid diagnostic test, relatively inexpensive and accurate (Nielsen *et al.,* 2004).

Serodiagnonostic methods for brucellosis have primarily been based on serology with lipopolysaccharides (LPS) from smooth strains producing greatest immunological response (Kittelberger *et al.,* 1997). In this study, different serological tests were applied on the serum samples; RBT gave 64, SAT gave 58 RT gave 55 and ELISA test gave 66 positive results (Table, 3). These findings agreed with (Verma *et al.,* 2000 and Nielsen

et al., 2005). The higher positivity of ELISA test generally has very high sensitivity and excellent screening assays for diagnosis of *brucella* especially in individual animal test of serum (Wright *et al.,* 1997 and Gall *et al.,* 2001 and McGiven *et al.,* 2003).

CONCLUSION

Finally, we conclude that in order to eradicate and control brucellosis, we must apply good surveillance reliable diagnostic test. The practical serological diagnosis must be based on screening test of high sensitivity followed by a confirmatory test as ELISA test of high specificity. A combination of serological test with DFAT is usually needed for diagnosis of *brucella* organisms in aborted cases.

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مقارنة بعض الطرق السريعة لتشخيص مرض البروسيلا فى الأبقار المجهضة مع الطريقة التقليدية

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تم تجميع عدد 158 عينة من أبقار مجهضة اشتملت على عدد 102 عينة أفرازات رحمية وعدد 56 عينة السائل النفاسى وتم أخذ عينات دم من نفس الأبقار المجهضة لفحصها سيرولوجيا لمرض البروسيلا. تم زرع هذه العينات بكترولوجيا على أطباق تحتوى على بروسيلا أجار مضافا إليها نسبة 10% دم أغنام وحفظت هذه الأطباق في درجة حرارة 37 درجة مئوية في حضانة تحتوى على نسبة 5 % من غاز ثاني أكسيد الكربون لمدة 72-120 ساعة. تم أخذ المعزولات الايجابية من الاطباق لاجراء التجارب البيوكيميائية عليها للتعرف على نوع البروسيلا المعزول وتم تطبيق اختبار الدوت اليزا على العينات الايجابية للتأكد من النتائج. وكذلك تم تطبيق الاختبار الفلورسينتى المباشر على عينات الأبقار المجهضة بتثبيتها على الشرائح الخاصة بالاختبار الفلورسينتى ووضع صبغة الفلورسين التى تحتوى على المضادات لميكروب البروسيلا وفحصها تحت الميكروسكوب الفلورسينتى باستخدام الأشعة فوق البنفسجية. وكذلك تم تطبيق الاختبارات المصلية على مصل هذه الأبقار المجهضة مثل اختبار التلزن البطيء والروز بنجال والريفانول وكذلك اختبار الاليزا. وكانت نتائج العزل كالتالى: عزل ميكروب البروسيلا ميليتنسيز من عدد 12 عينة بنسبة 7.59% من اجمالى عينات الأبقار المجهضة تشمل عدد 8 عينات ايجابية بنسبة 7.84% من الافرازات الرحمية وعدد 4 عينات ايجابية بنسبة 7.14% من السائل النفاسى وكانت نتيجة اختبار الدوت اليزا نفس النتائج. أما نتيجة الاختبار الفلورسينتى المباشر كانت ايجابية لعدد 25 بنسبة 15.82% لعدد 16 عينة ايجابية بنسبة 15.69% من الافرازات الرحمية وعدد 9 عينات ايجابية بنسبة 16.07% من الأنسجة النفاسية. وكانت نتيجة الاختبارات المصلية كالآتى: عدد 64 عينة ايجابية لاختبار الروزبنجال بنسبة 40.51 % ، عدد 58 عينة ايجابية باستخدام اختبار التلزن البطئ بنسبة 36.71 % ، وعدد ٥٥ عينة ايجابية لاختبار الريفانول بنسبة 34.81 % وكانت نتيجة اختبار الاليزا هو عدد 66 عينة ايجابية بنسبة 41.77 %. وأخيرا لكى نتخلص ونكافح مرض البروسيلا لابد من تطبيق الاختبارات الحقيقية والتشخيص المصلى الفعال وأن يعتمد على اختبار كاشف سريع مباشر يتميز بالكفاءة العالية مع استخدام اختبار تأكيدى مثل الاليزا لما يتميز به من دقة فى التشخيص بجانب الاختبارات السيرولوجية السريعة الكاشفة وكذلك يوصى باستخدام الاختبار الفلورسينتى المباشر للتشخيص السريع لحالات الاجهاض فى الابقار.