EVALUATION OF THE LOCALLY PREPARED ROSE BENGAL PLATE ANTIGEN OF *BRUCELLA ABORTUS* STRAIN 19

ABD-EL-AZEEM, K. A., SALWA S. AWAD AND K. EL-DEEN HUSSEIN

Veterinary Serum and Vaccine Research Institute, ARC, DOKKi- GIZA – EGYPT

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Abstract

Rose Bengal antigen was prepared from vaccinal *Brucella abortus* strain 19 (S-19 RB Ag.). The effectiveness of this antigen was evaluated and compared with the standard Rose Bengal antigen which is prepared from *Brucella abortus* strain 99 (S-99 RB Ag.).Evaluation was done by using blood sera from vaccinated and experimentally infected guinea-pigs and from suspected naturally infected cattle, camel, sheep and goats.

The results revealed that, both antigens have nearly similar sensitivity and specificity rates when they were used in testing the sera of suspected cases of different animals .However, S-19 RB Ag had higher sensitivity rate than S-99 RB Ag when used with sera of vaccinated guinea-pigs.

INTRODUCTION

Brucellosis is still one of the most important highly contagious diseases affecting all farm and domestic animals in most countries of the world. It is considered by WHO (1992) as the most wide spread zoonosis. Animal brucellosis causes highly economic losses due to reproductive failure such as abortion, or birth of unthrifty newborn in cattle, and orchitis, epididymitis with frequent sterility in male .Persistent infection is a characteristic of this intracellular organisms with shedding in the reproductive and mammary secretions (Brenner *et al.*, 2005), and so, it contaminates the environment. An effective control of animal brucellosis depends on the identification of the disease by conventional serological tests and compulsory slaughter of low or in non-infected region where the infection has been newly introduced.

Tube agglutination test has been the principal conventional serological test used for diagnosis of brucellosis. This test has serious defects in both sensitivity and specificity (Alton *et al.,* 1988). Such defects have initiated many investigators to develop new antigens such as buffered Brucella antigen.

The Rose Bengal test (RBT) is a simple spot agglutination test which is effective in the diagnosis of animal and human brucellosis where it is used as screen and definitive test (Alton *et al.*, 1988). The standard Rose Bengal antigen has been produced from freeze dried original seed culture of smooth *Brucella abortus* strain 99 (S-99) in Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt since 1990 (Refai *et al.,* 1990), but, the successive subculturing of this strain for uncountable times in order to prepare Rose Bengal antigen may lead to be changed in

some phenotypic and or even genotypic characteristics (Fitch *et al.,* 1990) which affect antigen production quality.

Smooth strain of *Brucella abortus* strain 19(S-19) could be used as a replacement of S-99 in preparing Rose Bengal antigen (Alton *et al.,* 1988).

The aim of this study is a trial to prepare Rose Bengal antigen from *Brucella abortus* strain 19 (S-19) as alternative to *Brucella abortus* strain 99 (S-99), and evaluate the effectiveness of this new antigen in comparison with standard currently used S-99 Rose Bengal antigen in sera of vaccinated and experimentally infected guinea-pigs, and sera of cattle, camel, sheep and goats suspected to be infected with brucellosis.

MATERIALS AND METHODS

1- Brucella strains

1-1-Virulent *Brucella abortus* strain 2308 (S-2308) was obtained from Vet.Lab, NewHow, Surry, K.T.15 England. This strain was used for experimental infection of guinea pigs.

1-2- Brucella melitensis biovar 1 field strain (locally isolated). This strain was used for experimental infection of guinea pigs.

1-3-Brucella abortus strain 19 (S-19) was used for preparing Rose Bengal antigen.

The Brucella strains were grown in Roux-bottles of trypticase soy agar medium (Oxoid) at 37°C for 48 hours, the growth was harvested, and culture's purity was checked. The S-2308 and field strain were diluted by sterile normal saline to contain $5x10^3$ viable Brucella count /ml, while, the S-19 suspension was inactivated in a water bath at 90°C for 60 min. After cooling to room temperature, a sample of the Brucella suspension was checked for purity and sterility. (Alton *et al.*, 1988)

2- Brucella antigens

2-1- Standard locally prepared *Brucella abortus* strain 99 Rose Bengal antigen (S 99-RB Ag) was obtained from Veterinary Serum and Vaccine Research Institute, Abbassia.

2-2- *Brucella abortus* strain 19 Rose Bengal antigen (S-19 RBAg-19) was prepared from inactivated S-19 suspension according to Alton *et al.,* (1988)

3- **Brucella Vaccines**

3-1- Locally prepared Live *Brucella abortus* strain 19 (S-19) vaccine and Live *Brucella melitensis* Rev.1 (Rev.1) vaccine, were used for vaccination of guinea pigs.

4-**Animals and experiment schedule**

4-1- Guinea-pigs: 175 Brucella sero-negative guinea-pigs after being examined by Rose Bengal test and tube agglutination test of not less than 500 grams weight were used. These animals were divided to 5 groups, thirty - five guinea pigs in each group. Groups 1 and 2 were experimentally infected with $5x10^3$ viable organisms of *Brucella*

abortus S-2308 and *Brucella melitensis* field strain, respectively. Groups 3 and 4 were vaccinated by S-19 and Rev.1 vaccine, respectively. Each guinea pig was vaccinated with 1/15 of the bovine dose ($3\text{-}10x10^9$ viable organisms/1 ml dose) of S-19 vaccine and $1\text{-}5X10^9$/ml of Rev.1 vaccine according to British veterinary pharmacopoeia (1985).Group 5 was used as control negative group, and all guinea pigs were sacrificed after 8 weeks of vaccination and infection, and the spleen and blood sera of each animal were collected separately.

4-2- Farm animals: 148 aborted animals of which 9 cattle, 11 camels, 39 goats and 89 sheep were used. The stomach, fetal content, fetal membrane and parts of aborted fetus were collected, and also, 148 blood sera were collected from these aborted animals. The collected tissues were macerated and cultivated on serum trypticase soy agar medium for isolation of Brucella organisms. Bacteriological identification was done according to Corner *et al.* (1985), and the Brucella positive cases were confirmed by Brucella monospecific sera Anti-A and Anti-M (Vet.Lab, NewHow, Weybridge, Surry, K.T.15 England). The blood sera were examined by both RB antigens (S-99 RB Ag and S-19 RB Ag).The specificity and sensitivity of the antigens were measured according to the chart described by Ronald (1991) as illustrated in Table 1.Test sensitivity is defined as a likelihood of a positive test result in diseased animals known to have the disease (True positive rate). Test specificity is the likelihood of a negative result in diseased animals known tube free of disease (True negative rate).

RESULTS AND DISCUSSION

Brucellosis is a contagious disease in all animals. It is a disease of pregnant ruminants. It causes placentitis leading to death and expulsion of the fetus. Brucella organisms may often be recovered from a vaginal swab taken in six week following parturition or abortion, also, from parts of fetal membrane which usually contains numerous numbers of Brucella organisms. The most valuable materials from the aborted fetus used for cultural examination are the stomach contents, lung and spleen. Brucella organisms were isolated and identified from aborted fetus and vaginal swabs of suspected cases of farm animals, and also, from vaccinated and experimentally infected guinea pigs. Because the cultural examination for the diagnosis of the animal brucellosis was considered unrewandering, so, the serodiagnostic test may be necessary. The blood sera were examined by strain 99 Rose Bengal antigen (S-99 RB Ag) which was prepared from *Brucella abortus* strain 99 as standard antigen. The sensitivity and specificity of this standard antigen was compared with the newly prepared S-19 RB Ag.

The sensitivity percent of both antigens used in sera of infected guinea pigs (Table 2) was somewhat higher in animals infected with *Brucella melitensis* than those

infected with *Brucella abortus* (83.3 %and 77.4 %, respectively). This may be due to that *Brucella melitensis* is more pathogenic than *Brucella abortus* in guinea-pigs (Corbel, 1985). However, the sensitivity and specificity of both antigens were the same.

Concerning vaccinated guinea pigs (Table 3), it is clear that the sensitivity and specificity rates were the same in group vaccinated with Live (Rev.1) vaccine, but, guinea pigs vaccinated by live S-19 vaccine, did not induce distinct variation. The S-19 Rose Bengal antigen and standard antigen showed sensitivity rates of 78.9 % and 73.7 %, respectively. This represents a little variation which may be due to that prepared S-19 Rose Bengal antigen, may not contain any rough Brucella cells. It is known that under laboratory conditions, variations of Brucella organisms from smooth to rough colonies occurred especially when the strain was subcultured for many times (Seham, *et al.,* 2005).

In cattle, the Rose Bengal test is effective as rapid presumptive test, it may be oversensitive for diagnosis in individual cattle (Alton *et al.*, 1988). So, as shown in Table 4, both the S-19 Rose Bengal and standard one induce 100 % sensitivity and specificity (no false positive).It is emphasized here that the sensitivity of the Rose Bengal test is greatly influenced by the temperature at which the reaction takes place. As known, it should be at 22°C ± 4°C, as it is known that sera and antigens if used straight from the refrigerator will react poorly (Nicoletti, 1967). The standard Rose Bengal antigen which was prepared from *Brucella abortus* 99 has been used for diagnosis in sera of sheep and goats in the Canadian (Nielsen *et al.,* 2004) and in the European Union (Trittarelli, *et al.,*2005), as shown in Table 5. This test produced 100% specificity rate when tested with sera of the sheep and goats, and produced sensitivity nearly to 100%. Both antigens either S-19 Rose Bengal antigen or the standard one gave the same results as they induced 93.7% sensitivity of sheep sera and 96.4% of the goats sera .These results agreed with Ferreira, *et al. (*2003), as they found that the Rose Bengal test showed 100% specificity when testing the sera from 212 Brucella -free sheep and when they tested sera from 219 *Brucella melitensis* culture positive sheep; the standard Rose Bengal test induced 95% sensitivity. It is clear that, there is a great similarity of the specificity and sensitivity rates between the two antigens (S-19 Rose Bengal antigen and standard one) when they were used in suspected Brucellosis in domestic farm animals (Camel, Cattle, Sheep and Goats) or when they were used in experimentally infected guinea pigs. When these antigens were used with sera from vaccinated guinea pigs, the S-19 Rose Bengal antigen gave higher sensitivity rate than that induced by the standard Rose Bengal antigen.

So, it could be concluded that the S-19 Rose Bengal antigen which was prepared from *Brucella abortus* vaccinal strain 19 could be used as alternative to the standard Rose Bengal antigen which was prepared from *Brucella abortus* strain 99.

Table 1. Chart of the sensitivity and specificity of the serodiagnostic tests.

serodiagnostic tests	Isolation of Brucella organisms	
	positive	negative
Positive	True positive (TP)	False positive (FP)
Negative	False negative (FN)	True negative (TN)
	Sensitivity = $\frac{TP}{TP+FN}$	Specificity = $\frac{TN}{TN+FP}$

Table 2. The sensitivity and specificity of S-19 Rose Bengal antigen compared with Standard Rose Bengal antigen on experimentally infected guinea pigs.

Results of Rose Bengal test	Guinea pigs infected with *Brucella melitensis* field strain				Guinea pigs infected with *Brucella abortus* strain 2308			
	Isolated Brucella from the spleen		Brucella free spleen		Isolated Brucella from the spleen		Brucella free spleen	
	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag
Positive	25	25	1	1	24	24	1	1
Negative	05	05	4	4	07	07	3	3
Total	30	30	5	5	31	31	4	4
Sensitivity	25/30 (83/3%)				24/31 (77.4%)			
Specificity			4/5 (80%)				3/4 (75%)	

Table 3.The sensitivity and specificity of S-19 Rose Bengal antigen compared with standard antigen on vaccinated guinea pigs.

Results of Rose Bengal test	Vaccinated guinea-pigs with S-19 vaccine				Vaccinated guinea-pigs with Rev-1 vaccine			
	Isolated Brucella from spleen		Brucella free spleen		Isolated Brucella from spleen		Brucella free spleen	
	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RB Ag	Standard RB Ag
Positive	15	14	04	04	15	15	04	04
Negative	04	05	12	12	05	05	11	11
Total	19	19	16	16	20	20	15	15
Sensitivity	15/19 (78.9%)	14/19 (73.7%)			15/20 (75%)			
Specificity			12/16 (75%)				11/15 (73.3%)	

Table 4.ensitivity and specificity of S-19 Rose Bengal antigen compared with standard Rose Bengal antigen on naturally infected cattle and camel.

Results of Rose Bengal test	camel				cattle			
	Isolated Brucella from the spleen		Brucella free spleen		Isolated Brucella from the spleen		Brucella free spleen	
	S-19 RB Ag	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RB Ag	Standard RB Ag
Positive	7	7	-	-	6	6	-	-
Negative	-	-	4	4	-	-	3	3
Total	7	7	4	4	6	6	3	3
Sensitivity	7/7 (100%)				6/6 (100%)			
Specificity			4/4 (100%)				3/3 (100%)	

Table 5.Sensitivity and specificity of S-19 Rose Bengal antigen compared with standard Rose Bengal antigen on naturally infected sheep and goats.

Results of Rose Bengal test	goats				sheep			
	Brucella Isolated from the spleen		Brucella free spleen		Brucella Isolated from the spleen		Brucella free spleen	
	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag	S-19 RBAg	Standard RB Ag
Positive	27	27	-	-	60	60	-	-
Negative	01	01	11	11	04	04	25	25
Total	28	28	11	11	64	64	25	25
Sensitivity	27/28 (96.4%)				60/64 (93.7%)			
Specificity			11/11 (100%)				25/25 (100%)	

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تقييم أنتيجين الروزبنجال المحضر محلياً من البروسيلا أبورتس عترة ١٩

خالدعبدالقادر عبد العظيم ، سلوىسعدعوض ، كمال الدين حسين

معهد بحوث الأمصال و اللقاحات البيطرية بالعباسية- مركز البحوث الزراعية – وزارة الزراعة- الدقى- الجيزة- مصر

تم تحضير أنتيجين الروزبنجال من البروسيلا أبورتس عترة ١٩ وتم تقييم كفاءة ذلك الأنتيجن الجديد بالمقارنة بأنتيجين الروزبنجال العيارى المحضر من البروسيلا أبورتس عترة ٩٩ وذلك باختبار مصل دم مجموعات مختلفة من الأرانب الهندي المحصنة بلقاح البروسيلا أبورتس عترة ١٩ ولقاح البروسيلا ميليتنسس ريف-١ وأخرى أخذت العدوى تجريبياً وأيضاً من مصل ماشية وإبل وأغنام وماعز محتمل إصابتها بمرض البروسيلا. ولقد وجد أن هناك تشابهاً كبيراً في معدل حساسية وخصوصية كلا الأنتيجينين عند فحص أمصال دم حيوانات المزرعة والأرانب الهندي المعداه تجريبياً بينما أعطى أنتيجين الروزبنجال المحضر من العترة ١٩ درجة حساسية أكبر من الأنتيجن العيارى المحضر من العترة ٩٩عند فحص مصل الأرانب الهندي المحصنة بلقاح عترة ١٩ . لذا يتضح أن البروسيلا أبورتس ١٩ يمكن إستخدامها كبديل لعترة البروسيلا أبورتس ٩٩ في إنتاج أنتيجين الروزبنجال.