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EVALUATION OF TRIALS OF VACCINATION AND REVACCINATION OF CATTLE USING RB51 AND S19 VACCINES BY MONITORING OF THE SEROLOGICAL RESPONSE AND SHEDDING OF THE VACCINAL STRAINS

(With 4 Tables)

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تقييم محاولات تحصين الأبقار لمرض الأجهاض المعدى باستخدام العترة S19 والعترة RB51 لميكروب الأجهاض المعدى عن طريق قياس رد الفعل المناعى واقراز العترة اللقاحية من الأبقار المحصنة

رجب عزام على

تم فى هذه الدراسة تقييم ثلاثة محاولات لتحصين الابقار ضد مرض الاجهاض المعدى "البروسيلا" على اساس متابعة رد الفعل المناعى وعزل العترات اللقاحية من الابقار المحصنة. لاجراء هذه الدراسة تم تقسيم عدد ٨٤ بقرة "فريزيان" الى ثلاثة مجموعات تكونت الاولى منها من عدد ٤٢ بقرة تم تحصينها باستخدام العترة RB51 من ميكروب الاجهاض المعدى عند عمر ٥-٨ شهور ثم اعيد التحصين بنفس العترة عند عمر ٢٠-٢٢ شهرا. وقد تم تحصين حيوانات المجموعة الثانية وعددها ٢٤ بقرة باستخدام العترة S19 عند عمر ٥-٨ شهور وأعيد التحصين بالعترة RB51 عند عمر ٢٠-٢٢ شهرا. أما الأبقار فى المجموعة الثالثة فكان عددها ١٨ بقرة وتم تحصينها عند عمر ٥-٨ شهور وباستخدام العترة S19 فقط. وقد وجد ان مصل الدم لجميع أبقار المجموعة الأولى أظهر تفاعلا ايجابيا ضد المولدات الخشنة بداية من الأسبوع الثانى بعد التحصين واستمر هذا التفاعل الأيجابى حتى الأسبوع التاسع عشر بعد التحصين فى بعض الأبقار علما بان جميع أبقار المجموعة قد اظهرت نتيجة سالبة عند اختبارها ضد المولدات الملساء. بالنسبة لحيوانات المجموعة الثانية فقد اظهرت الحيوانات المحصنة باستخدام العترة S19 ايجابية للأختبارات التقليدية لمرض الأجهاض المعدى منذ الأسبوع الثانى بعد التحصين واستمرت هذه الأيجابية حتى الأسبوع الثامن والعشرين بعد التحصين. أما الحيوانات التى تم اعادة تحصينها باستخدام العترة RB51 فى هذه المجموعة فقد كانت ايجابية عند اختبارها ضد المولدات الخشنة حتى ٢٢ اسبوعا بعد التحصين بينما كانت سلبية لأختبارها بالمولدات الملساء. فيما يختص بحيوانات المجموعة الثالثة فقد وجد انها سالبة للاختبارات التقليدية بعد عام من تحصينها باستخدام العترة S19. أما بالنسبة للفحص الميكروبى فقد أمكن عزل العترة اللقاحيةRB51 من اللبن من بقرة واحدة فقط بعد الولادة بثلاثة ايام كما تم عزل نفس الميكروب من الأفرازات المهبلية لبقرتين أخريتين جميعها فى المجموعة الأولى بينما لم يمكن عزل الميكروب من الأبقار التى تم تحصينها

كعجلات باستخدام العترة S19 واعيد تحصينها عند عمر ٢٠-٢٢ شهرا باستخدام العترة RB51.

SUMMARY

In this study, evaluation of three trials of vaccination of cattle against brucellosis through monitoring of the serological immune response and shedding of the vaccinal strains was carried out. For this purpose, a total of 84 Friesian cows were divided into three groups. The first group consisted of 42 cows that were vaccinated at the age of 5-8 months with *Brucella abortus* SRB51 vaccine and then revaccinated at the age of 20-22 months with the same vaccine. Animals of the second group (24 cows) were vaccinated at the age of 5-8 months with *Brucella abortus* S19 then revaccinated with RB51 vaccine at the age of 20-22 months, while animals of the third group (18) were vaccinated only at the age of 5-8 months with *Brucella abortus* S19. Sera of all vaccinates of the first group reacted positively at the second week post vaccination and some animals continued up to 19 weeks post vaccination using rough antigen. Employing smooth antigen, the conventional tests showed negative results. Sera of S19 vaccinated animals seroconverted at the 2nd week post vaccination and some continued up to 28 weeks post vaccination using the conventional tests. RB51 revaccinated animals in this group developed antibodies against the rough antigen up to 22 weeks post vaccination and no antibodies against the smooth antigen were detected. Examination of animals that were vaccinated only using S19 at 5-8 months after one year revealed that all animals were serologically negative. Bacteriologically, one cow from the first group shed the RB51vaccinal strain in milk three days post parturition and two cows shed the organism in their vaginal discharges. Cows that were vaccinated as calves with S19 and revaccinated as adults with RB51 showed no organisms in their milk or vaginal discharges.

Key words: *Brucellosis, vaccination, serological response*

INTRODUCTION

Brucellosis is a worldwide serious disease. It still affects large numbers of animals in Egypt causing abortion and infertility. The disease is transmissible to occupationally exposed humans. Brucella organisms are Gram-negative facultative intracellular bacteria that infect macrophages where they persist and evade immune elimination, Covert

et al. (2005). Chronic infections are thought to be due to their ability to avoid the killing mechanisms within the host cells.

Prevention of bovine brucellosis is achieved by using live attenuated vaccines, Adone and Ciuchini (2001). Recently a new official calfhood vaccine; *Brucella abortus* S RB51 has been approved in the United States for use in brucellosis eradication programs. *Brucella abortus* SRB51 is a lipopolysaccharide O antigen-deficient mutant of the virulent strain 2308 of *Brucella abortus*, Schurig *et al.* (2002). It has been documented to be protective in cattle and not to induce antibodies that interfere with brucellosis serological surveillance tests which identify antibodies to liopopolysaccharides, Stevens *et al.* (1995) and Olsen *et al.* (1997).

In Egypt, *Brucella melitensis biovar3* remains the prevalent type of brucella affecting cattle, Salem and Hosein (1990), Hosein *et al.* (2002) and Soliman, H.S. (2006). Several efforts have been made to control the disease through the use of vaccines including *Brucella abortus* SRB51. The present study was carried out to evaluate some trials of vaccination and revaccination of cattle using RB51 and S19 vaccines. The serological immune response in cows vaccinated with *Brucella abortus* S RB51 (as calfhood vaccination and revaccinated with the same vaccine as adults) and cows vaccinated with *Brucella abortus* S19 (as calfhood vaccination and revaccinated with *Brucella abortus* SRB51 as adults) in the light of possibility of shedding the vaccinal strains were investigated.

MATERIALS and METHODS

1 – Animals: A total of 84 Friesian cows in Damitta Governorate were used in this study through the period (January 2004 – April 2006)

2 - Experimental design: Three groups of animals were randomly selected

Group I: A total of 42 cows were vaccinated at the age of 5-8 months with *Brucella abortus* SRB51 vaccine (3×10^9 S.C) and revaccinated with the same vaccine at 20-22 months of age (Most of these cows were pregnant at this time).

Group II: A total of 24 cows were vaccinated at the age of 5-8 months with *Brucella abortus* S19 (5×10^{10} S.C) and revaccinated with *Brucella abortus* SRB51 (3×10^9 S.C) vaccine at 20-22 months of age. (Also most of the cows were pregnant).

Group III: A total of 18 cows were vaccinated as calves of 5-8 months age with *Brucella abortus* S19 only.

3 – Samples:

a. Blood serum samples were collected from all cows after vaccination and revaccination weekly up to 30 weeks.

b. Colostrum and milk samples and vaginal discharges all from cows were collected at the 1st, 3rd and 7th days after parturition.

4 – Vaccines:

a. *Brucella abortus* SRB51 vaccine U.S.Vet. Licence No. 188 Professional Biological Company 4950 York street Denever USA.

b. *Brucella abortus* S19 vaccine; it was obtained from the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

5 - Serological tests:

a. Tube Agglutination Test (TAT), was carried out using smooth and rough antigens. The test was carried out according to Alton *et al.* (1988).

b. Rose Bengal Test (RBT), was carried out according to Morgan *et al.* (1978).

c. Buffered Acidified Plate antigen Test (BAPAT), was carried out according to Alton *et al.* (1988).

6 - Bacteriological examination:

Isolation, identification and typing of brucella organisms were carried out according to Alton *et al.* (1988)

RESULTS

Table 1: Monitoring the serological immune response of cows of group I

Used vaccine		Age of vaccination	Serological response	
			Smooth antigen	Rough antigen
Vaccination	RB51	5-8 months	-ve	+ve* 2-19 w.p.v
Revaccination	RB51	20-22 months	-ve	+ve 2-22 w. p.v

* All cows seroconverted w. p.v = weeks postvaccination

Table 2: Monitoring the serological immune response of cows of group II

Used vaccine		Age of vaccination	Serological response	
			Smooth antigen	Rough antigen
Vaccination	S19	5-8 months	+ve* 2-28 w.p.v	-ve
Revaccination	RB51	20-22 months	-ve	+ve 2-20 w.p.v

* All animals seroconverted w.p.v = weeks postvaccination

Table 3: Monitoring the serological immune response of cows of group III

Used vaccine		Age of vaccination	Serological response	
			Smooth antigen	Rough antigen
Vaccination	S19	5-8 months	+ve* 2-28 w.p.v	-ve

* All cows seroconverted

Re-examination after one year showed that all cows remained seronegative throughout the experiment

Table 4: Bacteriological findings.

Cow group	Shedding of the vaccinal strain		Abortion
	Milk	Vaginal discharges	
I	One cow +ve*	2 cows +ve**	-ve
II	-ve	-ve	-ve
III	-ve	-ve	-ve

* Sample of 3 days post parturition
** Samples of one and 3 days post parturition

DISCUSSION

The humoral immune response of RB51 vaccinated calves of group I, using rough antigen, revealed that sera of all vaccinates reacted positively at the 2nd week post vaccination. Seroconvesion continued in some calves up to 19 weeks post vaccination, Table (1). Such humoral immune response is directed primarily to the outer membrane proteins but not to the lipopolysaccharide-O antigen as reported by Schurig *et al.* (1991).

On the other hand, sera of vaccinated calves showed complete negative results using the conventional tests and employing smooth antigen, these tests detect only antibodies against lipopolysaccharide-O antigen which characterize only the smooth brucella strains while RB51is a rough mutant which is devoid of lipopolysaccharide-O chain, Stevens *et al.* (1995). Revaccination of these cows using the same vaccine at 20-22 months age revealed the same serological profile and antibodies against the rough antigen continued up to 22 weeks post vaccination.

The above mentioned data confirmed that both vaccination and revaccination of adult cows using RB51 does not induce detectable antibodies against the smooth antigen employing the conventional tests used for detection of brucella infection and consequently it does not interfere with surveillance programs.

Serological examination of S19 vaccinates of group II, using the conventional serological tests, revealed that all calves seroconverted at the 2nd week post-vaccination and some continued up to 28 weeks post-vaccination, Table (2). Revaccination of these animals at 20-22 months using RB51 vaccine of age resulted in development of antibodies against the rough antigen up to up to 22 weeks post-vaccination. On the other hand, antibodies against the smooth antigen could not be detected. The obtained results confirmed the lack of seroconversion of animals that previously vaccinated with S19 as calfhood vaccination when revaccinated with RB51 vaccine as adults indicating the safety of such procedure. RB51 vaccine was described as a safe procedure by Zambrano *et al.* (1995) and Palmer *et al.* (1997).

Cows of group III that were vaccinated using S19 as calves of 5-8 months showed the same pattern of calves of group II. Examination of these animals after nearly one year revealed that all cows maintained their negative serological status when became adults.

Evaluation of the trials employed in this study on bacteriological basis, Table (4), showed that among the cows of group I, one cow shed RB51 vaccinal strain in milk which could be detected only from the sample collected 3 days post-parturition and two cows shed the organism one and 3 days post-parturition in their vaginal discharges including the cow whose milk was bacteriolgically positive.

This indicates that RB51 can be shed in milk or vaginal discharges of adult RB51 vaccinated cows especially when they are vaccinated during pregnancy. Shedding of RB51 vaccinal strain in vaginal discharges was reported by Samartino *et al.* (2000) and Hosein *et al.* (2005). Shedding in milk was also reported by Samartino et al. (2000) who suggested that shedding of RB51 in milk can actually benefit herd immunity due to ingestion of these organisms by young calves to induce protective immunity.

On the contrary, such finding may lead me to believe that such situation may be hazardous from the epizootiological as well as the epidemiological points of view as it may lead to cases of latent infection in calves due to ingestion of the organism in milk as well as it may result in human infection that will be difficult to be diagnosed by the current

conventional tests which are designed to detect infection by smooth brucella strains.

On the other hand, concerning cows that were vaccinated with S19 as a calfhood vaccine and revaccinated as adults with RB51 vaccine, there was no shedding of the vaccinal strain neither in milk nor in vaginal discharges. Such results may be attributed to the protective immunity induced by the calfhood S19 vaccination.

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