

Serum Antibody Responses of Different Age Groups of Sudanese Zebu Cattle to *Brucella abortus* Strain 19 Vaccines

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ملخص البحث

تم تقييم الاستجابة المصلية للأجسام المضادة (الضد) في أبقار سودانية ذات أعمار مختلفة ، نتيجة لتحصينها بمستحضرين من لقاح العترة 19 للبروسيلة المجهضة باستخدام اختبارات الـ روزينقال الصحنى ، تثبيت المتممة ، التراص الأنبوبي ، اللبني الحلقي و هي اختبارات تقليدية لتحديد مستوى الضد في المصل ، علاوة على اختباري الـ إيليزا غير المباشرة و التنافسية. عند استخدام أحد المستحضرين في تحصين مجموعتين من عجول عمرها عامين ، الأولى بالجرعة الكاملة و الثانية بالجرعة المخفضة (1/10 من الجرعة الكاملة) ، وجد أن أعلى متوسط للضد باختبار التراص هو 1039.3 و 256.5 وحدة عالمية/مل على التوالي. لقد انخفض مستوى الضد إلى دون المستوى التشخيصي بالاختبارات التقليدية بعد 164 و 73 يوماً من التحصين للمجموعتين على التوالي. عند تحصين بقرتين (عمر 10 أعوام ووزن 200 كيلو جرام) بالجرعة المخفضة من نفس اللقاح وحقنهما في نفس الوقت بعقار استيورت الـ ديمنازين (برنيل) بلغ أعلى متوسط للضد المتلزن 307.6 وحدة عالمية/مل و انخفض إلى دون الحد التشخيصي بعد 35 يوماً من التطعيم ولكن ظلنا موجبتان لاختبار تثبيت المتممة احدهما لليوم 45 و الأخرى لليوم 74 لتصبح سالبة في اليوم 105 بعد التحصين. عند تحصين بقرتين أخريين في عمر 14 عاماً ووزن 300 كيلوجرام بالجرعة المخفضة لنفس اللقاح وجد أن أعلى متوسط للضد المتلزن 635 وحدة عالمية/مل و انخفض إلى دون الحد التشخيصي في اليوم 164 بعد التحصين. عند استعمال المستحضر الثاني لتحصين مجموعتين من العجول في أعمار 5-7 و 12-14 شهراً بالجرعة الكاملة، وجد أن أعلى متوسط للضد المتلزن قد بلغ 1108 و 862 وحدة عالمية/مل/مصل على التوالي. و انخفض إلى المستوى السالب في يومي 100 و 85 لتحصين المجموعتين على التوالي. أما بالنسبة لبقرتين أخريين في عمر 10 أعوام و اللتين حصنتا بالجرعة المخفضة لهذا اللقاح الثاني أيضاً ، فقد بلغ أعلى متوسط للضد المتلزن 1723.5 وحدة عالمية/مل ، و انخفض إلى دون الحد التشخيصي في اليوم 105 بعد التحصين. ظلت إحدى البقرتين موجبة لاختبار اللين الحلقي حتى اليوم 31 بعد التحصين. وجد بعد مضي 960 يوماً من التحصين أن 4 من عجول حصنت بالجرعة الكاملة و المخفضة و بقرة حصنت باللقاح الأول قد اعطت نتيجة موجبة لاختباري الـ إيليزا ، بينما اظهرت إحدى البقرتين من اللتين حقننا بعقار البرنيل نتيجة سالبة لاختبار الـ إيليزا التنافسية. نوقش الاختلاف في الاستجابات المختلفة السابقة ووضع مقترح لتحصين الأبقار السودانية بأعمارها المختلفة بواسطة لقاح العترة 19 للبروسيلة المجهضة تحت ظروف الرعي التقليدية .

Summary

Serum antibody responses of Sudanese cattle of different age groups were assessed following their immunization with two *B. abortus* strain 19 vaccines. The conventional serological tests (RBPT, CFT, SAT, and MRT) for brucellosis, indirect and competitive ELISAs (iELISA and cELISA) were used. Using the first vaccine, the highest mean SAT antibody levels of two years old calves immunized with standard and reduced doses of the vaccine were 1039.3 i.u/ml and 256.5 i.u/ml, respectively. They reacted below diagnostic levels to the conventional

tests on day 164 and 73 p.v., respectively. The highest mean SAT antibody level of 10 years old and 200kg body weight cows vaccinated with the reduced dose of the vaccine and inoculated simultaneously with diminazene aceturate was 307.6 i.u/ml, which receded to negative status on day 35. These two cows were both positive for CFT until day 45 and one of them on day 73; the latter became negative on day 105. The highest mean SAT antibody level of 14 years 300 kg body weight cows immunized with the reduced dose was 635.6 i.u/ml. They reacted below diagnostic levels to the conventional tests on day 164. Using the second vaccine, the highest mean SAT antibody levels of 5-7 and 12-14 months old calves that were vaccinated with the full doses were 1108 and 862 i.u/ml., respectively. These titres receded to negative status on days 100 and 85, respectively. The highest mean SAT antibody level of cows over 10 years old, which were vaccinated with reduced doses of the vaccine, was 1723.05 i.u/ml.; this titre receded to a negative value on day 105. One cow of the latter group was positive for MRT till day 31. Variations in response to vaccination are discussed. On day 960 p.v. four calves vaccinated at two years of age with full or reduced doses and one cow vaccinated with a reduced dose of the first vaccine were positive to the iELISA and cELISA, while the other vaccinated cow which was injected with the diminazene aceturate was negative to cELISA. Suggestions are made for a protocol of vaccination of different age groups of Sudanese cattle, under traditional farming system, with S19 vaccine.

Introduction

Bovine brucellosis is wide spread in the Sudan and affects cattle kept under both organized and traditional systems of husbandry. *B. abortus* the major cause of the disease (Musa, 1990; 1995), was also isolated from man, camels and goats from various areas of the country (Musa, 1995). Such a situation (Plommet, 1986) has necessitated the control of *B. abortus* infection in cattle by vaccination.

At the present time, S19 vaccine is more reliable for the immunization of cattle against bovine brucellosis (Plommet, 1986). The vaccine affords 65-70% protection rate (Nicolotti, 1990) and may reduce individual and herd infections by 80% and 20%, respectively (Worthington *et al.*, 1974). Originally, S19 vaccine was meant to immunize calves during the first year at slightly varying restricted period of calthood (Cotton *et al.*, 1934; Nicoletti, 1990; Report, 1986). The age restrictions are imposed because the serological response to immunization is less prolonged in young animals and takes time

to disappear before they grow old enough to need testing. The agglutinin titres following administration of S19 vaccine can not be distinguished from those that result after virulent infection (King and Frank, 1961); a major disadvantage of S19 vaccine particularly in calves vaccinated after the eighth month (Wagela and Philpott, 1976). But, circumstances arise when immunization of adult cattle may be advisable and this necessitate the use of reduced doses of S19 (Report, 1986). Accordingly, two dose values of S19 vaccine are in common use; the standard, or full, dose for immunization of calves and the reduced dose for adult cattle; the latter has an additional advantage of much reduced serological response to vaccination (Alton *et al.*, 1984).

Susceptibility to *B. abortus* infection appears to be more strongly associated with sexual maturity than age (Nielsen and Duncan, 1990). The Sudanese zebu cattle (*Bos indicus*) reach maturity at about three years and calf between four and five years. Apparently, no attempts were made to study their serum antibody responses to vaccination with standard or reduced doses of S19 vaccine.

This study was initiated to compare persistence of brucella antibody titres in the Sudanese cattle following their immunization with either standard or reduced doses of S19 vaccine and to assign the doses for animals over eight months old accordingly.

Material and Methods

Vaccines:

Two *B. abortus* S19 vaccine manufactured by two different producers were used. The standard and reduced doses of vaccination, 3×10^{10} and 3×10^9 cfu, respectively, were inoculated subcutaneously.

Experimental animals:

Thirty-two unvaccinated Sudanese zebu cattle reared on the range in South Darfur State, ranged in age from five months to 14 years and were negative for brucellosis following screening by the Rose Bengal Plate Test (RBPT), were used in this study. These cattle belonged to Baggara, Kenana and Butana types and their crosses.

Experiment design:

Two calves, 5-7-months-old, were left as unvaccinated controls and the remaining 30 cattle were randomly divided into three groups as follows:

a) Group 1:

This group consisted of 11 animals obtained from herds that were found to be free from brucellosis and were vaccinated with the first vaccine. They were divided into five subgroups. Those of subgroups 1 and 2 were two-years-old calves of Baggara type. Subgroup 1 consisted of three calves which were vaccinated with a standard dose of the vaccine each. Subgroup 2 consisted of two calves, each was vaccinated with a reduced dose of the same vaccine. Subgroups 3, 4 and 5 consisted of six cows, two in each subgroup. Cows of subgroups 3 and 4 were Baggara type and each was about 10-years-old and 200 kg body weight. Those of subgroup 5 were Kenana and Butana types, over 14-years-old and of 300 kg body weight. All the six cows received reduced vaccinal doses, besides those of subgroup 4 were simultaneously inoculated with a curative dose of Diminazene aceturate; a trypanocidal drug which has a brucidal action as indicated by the producer (Fatro s.p.a Pharmaceutical Veterinary Industry, Ozzano, Enjilia (Bolongna) Italy).

b) Group 2:

Ten calves, 5-14-months-old and brought from brucella infected herd, were divided into two lots. The first consisted of four calves, 5-7 months-old and the second of six calves, 12-14-months-old. The 10-calves were each vaccinated with a full dose of the second vaccine.

c) Group 3:

The nine cattle of this group were adults and over 10-years-old and raised in a brucella infected herd. They were each vaccinated with a reduced dose of the second vaccine.

Assessment of responses to immunizations:

Blood samples were weekly collected from each of the 32 cattle during the first month and then Monthly till day 200 post vaccination (p.v.). In case of group 1 cattle, further samples were collected from animals that survived for up to day 960 (32 months) post immunization (p.i). In case of group 3 cattle collection of samples was continued after day 200 on monthly basis till they became negative to MRT.

Serum samples from group 1 cattle, were examined for *B. abortus* antibodies by the RBPT, SAT and CFT performed as described by Morgan *et al.* (1978). Samples collected after 32 months were examined by the above-mentioned conventional methods and by iELISA and cELISA performed as described by Brew *et al.* (1992). Samples from cattle of groups 2 and 3 were monitored for antibody responses by the RBPT, SAT and MRT for reasons discussed afterwards.

The SAT titers were expressed in international units per ml (i.u/ml.). Titers ≥ 100 were considered positive for vaccinated cattle and those below that were negative (Report, 1964).

During the course of this study, vaccinated animals were observed for principal manifestations of brucellosis like abortion, stillbirth, retention of placenta and infertility.

Results

The cattle used as unvaccinated controls remained negative for brucellosis throughout the study. No ill effects attributable to the disease were observed in the vaccinated cattle.

Antibody responses in cattle of group 1:

The mean SAT antibody levels of group 1 cattle and a comparison between their five subgroups are shown in Fig. 1.

Subgroup 1:

The mean SAT antibody level of the two-years-old calves vaccinated with full doses of the S19 vaccine reached a maximum titre of 1039.3 i.u/ml on day 14. It remained above diagnostic value for vaccinated cattle up to day 60, fluctuated below and above that level till day 164 then receded to a negative status and remained so up to day 200. The CFT antibodies were positive till day 105, variable by day 136 and negative on day 164. After 960 days (32 months) cattle of this subgroup were marginally positive for cELISA and iELISAs (Table 1).

Subgroup 2:

The highest mean SAT antibody titre, 256.5 i.u/ml., was recorded on day 14, fell to suspicious levels (77 - 87.3 i.u/ml.) on days 38 to 73 before returned to the negative status and likewise continued till day 200. The CFT antibodies were positive up to day 42 variable by day 73 and negative after that. At the 32nd month p.v. the animals were still marginally positive to cELISA (Table 1).

Table 1: Examination of individual cattle sera collected at the 32nd month following vaccination with *B. abortus* S19 vaccine

No.	Experimental Group	Vaccinal dose	RBPT	SAT	Indirect ELISA	Competitive ELISA
1	Gp 1 sub/Gp 1	Full dose	-	-	+	+(46.05%)*
2	Gp 1 sub/Gp 1	Full dose	-	-	+	+(57.6%)*
3	Gp 1 sub/Gp 1	Full dose	-	-	+	+(74.6%)*
4	Gp 1 sub/Gp 2	Reduced dose	-	-	+	+(70.5%)*
5	Gp 1 sub/Gp 4	Reduced dose	-	NT	NT	-
6	Gp 1 sub/Gp 4	Reduced dose	-	-	+	+(60.7%)*

Key: * the percentages denote to the values of the optical densities (OD) of the test samples over that of the negative control x 100., - = Negative for *B. abortus* antibodies, + = Positive for *B. abortus* antibodies., NT= not tested, Gp = Group, Sub/Gp= subgroup

Subgroup 3:

The highest mean SAT antibody titre, 128.3 i.u/ml., was reached at day 14. The titres receded to a negative status of 51.5 i.u/ml. by day 30, fluctuated below and above the diagnostic level at titres between 46.3 and 123 i.u/ml from day 42 to day 73 before they became negative and remained so up to day 200. The CFT was positive till day 42, variable on day 73 and negative by day 105.

Subgroup 4:

The highest mean SAT antibody titre recorded was 307.6 i.u/ml. which receded to a negative level on day 35. The CFT was positive on day 45, variable on day 73 and negative on day 105. On day 960, one of the cows became negative to cELISA and the other marginally positive. It was noticed that the drug had affected the persistence of SAT and cELISA antibodies compared to the more persistent analogous antibodies of subgroups 3 and 5 (Fig. 1).

Subgroup 5:

The highest mean SAT antibody level was 635.6 i.u/ml. which was recorded on day 7. The mean titre was 102 i.u/ml. on day 42 and had thereafter fluctuated between suspicious values of 64 and 77 i.u/ml. from day 73 to 164 before it became negative and likewise remained till day 200 (Fig. 1). The CFT antibodies were positive on day 73, variable by day 105 and negative on day 136. The SAT and CFT antibodies of this subgroup persisted longer than those of subgroups 3 and 4 despite their heavier weight indicating that this response to the vaccine was age, and not weight, dependant.

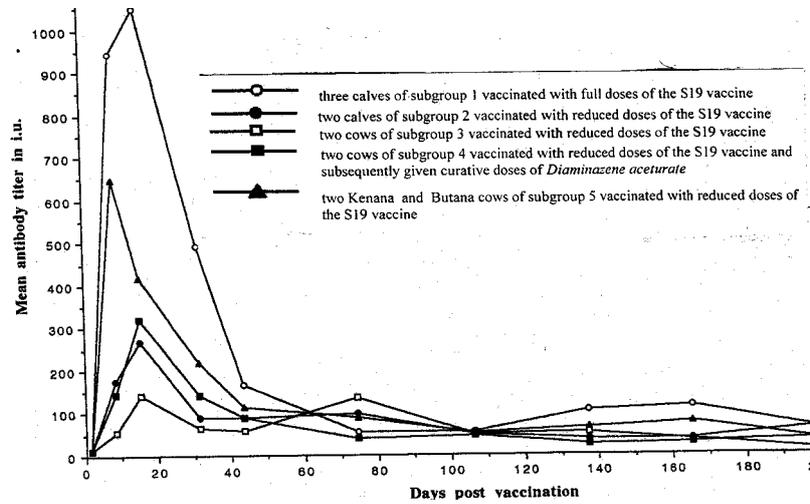


Fig. 1: Comparison between the five subgroups of cattle of group 1, vaccinated with full and reduced doses of the first Br. Abortus S19 vaccine

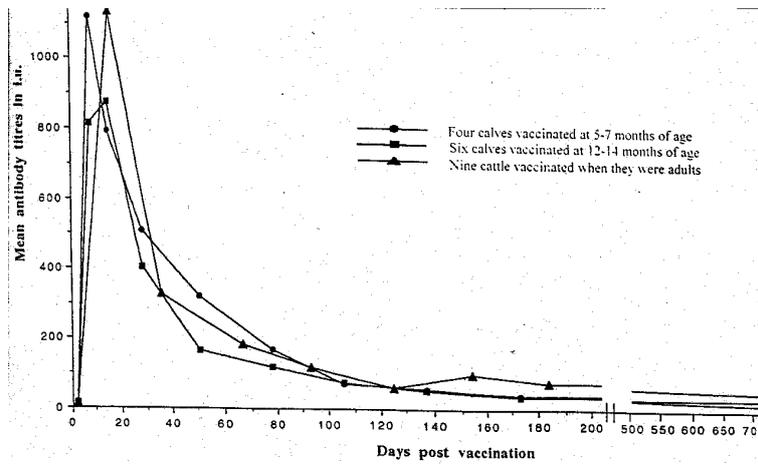


Fig. 2: Comparison between post vaccinal titers of two groups of calves at 5-7 and 12-14 months of age immunized with full doses of the second vaccine and adult cattle, which received reduced doses of the same product.

Antibody responses of groups 2 and 3 cattle:

Serum antibody responses of cattle of groups 2 and 3 and their comparison are shown in Fig. 2.

Group 2:**a) 5-7-months-old calves:**

The highest mean SAT antibody titre was 1108 i.u/ml. which was recorded on day six (Fig. 2). The mean titre remained above diagnostic level until day 100, then declined to a negative status that ranged between 11.5 and 57 i.u/ml. from day 106 to 200.

b) 12-14-months-old calves:

The highest mean SAT antibody titre was 862iu/ml which was recorded on day 13. The mean titre remained above diagnostic level till day 85 then receded to negative levels from day 85 to 200 (Fig. 2). Obviously 5-7-months-old calves responded better and quicker to the vaccine and their mean titre persisted longer than that of the 12-14-months-old ones. Antibodies of both groups receded to the same negative levels from day 106 to 200. After day 500 and up to day 750 the mean negative level of older calves was higher than that of the younger ones. The antibodies responses of the younger calves were attributed to a possible previous recent sensitization to *Brucella* organisms.

Group 3:

The antibody responses of this group are shown in Fig. 2. The mean SAT antibody titre reached its highest level (1723.05 i.u/ml.) in 14 days p.v. The mean titre remained above diagnostic level till day 105, fell to suspicious levels in day 121-180 before receded to a negative level (51.1 i.u/ml.) on day 200. One cow was MRT positive from day seven till day 31. Serum antibody responses to the second vaccine were higher than those to the first vaccine.

Discussion

It was difficult to obtain enough cattle at the specified ages for this study. As a result unequal and limited numbers were used. Although two S19 vaccines from different sources were employed, it was not meant to compare them. Serum antibody response of group 1 cattle was examined by the RBPT, SAT, CFT, iELISA and cELISA. But, in cases of groups 2 and 3 only RBPT, SAT and MRT were used because facilities for the other tests were not available. However, RBPT was found to be a suitable substitute for

CFT under such circumstances (Waghela *et al.*, 1980). Moreover, a close agreement between the results of RBPT and CFT does exist (Morgan *et al.*, 1969). SAT is also a suitable test under such conditions, because IgM persists in animals immunized with S19 vaccine and the SAT is more sensitive to this immunoglobulin isotype than to any other isotype, whereas CFT is particularly sensitive to IgG (Report, 1986).

Nielsen and Duncan (1990) have found that SAT antibodies appeared within five to seven days following vaccination with a standard dose of S19 vaccine, reached a peak of around 2000i.u/ml. at 2-3 weeks, and that 80% of animals vaccinated at 6 to 9 months of age had diagnostically significant levels within 12 months. In this study, SAT antibodies reached mean peaks (862 and 1108 i.u/ml.) in six to 14 days and receded to negative status between 3 and 5.5 months. But, the highest mean peak of SAT antibody responses of the Sudanese cattle were within the values stated by the aforementioned authors despite their references to results obtained from different cattle breeds. In Argentina, however, Santos *et al.* (1975) have found that young zebu heifers vaccinated at 6-8, 8-10 and 10-12 months did not reach a 100% negative status for up to 600 days post vaccination.

The two years old calves vaccinated with the reduced dose produced titres of serum antibodies lower than those of adult cattle which received similar doses, indicating that they were rather tolerant to the lower doses of the vaccine. Accordingly, two years old calves reared under traditional farming system in the Sudan can be immunized with the standard dose of S19 vaccine but they should be marked upon

vaccination so as to be identified during subsequent tests as in the case of adult cattle (Plommet, 1986).

Adult cattle, immunized with reduced doses, receded to negative status for SAT and CFT antibodies within 2.5 to 3 months. Similar results have previously been reported (Alton *et al.*, 1984). Hence, vaccination of adult cattle may be carried out without much persistence of agglutinins.

Antibody titres of two-years-old calves vaccinated with standard dose and those of adult cattle which received the reduced one, fluctuated between below and above diagnostic levels before returning

to negative status, but those vaccinated at 5-14 months of age didn't. These differences were attributed to age at vaccination (King and Frank, 1961). Variation in magnitude of antibody level produced by cattle which had received the same dose of S19 vaccine (groups 1, 2 and 3) has been ascribed to age at vaccination, dosage, method of administration and state of pregnancy (Nicoletti, 1990). Moreover, Nielsen and Duncan (1990) have observed that immunization of animals not previously sensitized to brucella is followed by low SAT and CFT responses which soon become negative. This observation also explains the reasons of quick response and rather long persistence of SAT antibodies in calves and cattle raised in a brucellosis positive farm (groups 2 and 3).

Previously, assessments of post vaccinal antibody titres was carried out by the conventional serological tests. However, these were superseded by ELISA tests. The fact that some cattle in this study remained iELISA and cELISA positive for 960 days p.v. makes the use of S19 vaccine more disadvantageous because of the difficulty in the discrimination between antibodies subsequent to vaccination and those due to natural infection. Fortunately, an ELISA test which discriminates between the two antibodies was developed (Mac Millan *et al.*, 1998). Moreover, to overcome the limitations of the use of S19 vaccine, a new non-agglutinogenic *B. abortus* attenuated RB51 vaccine was approved for conditional use in the USA (Report, 1999). Consequently, *B. abortus* S19 vaccine will still be in global use for a while.

In South Darfur State, it is a common practice to administer Diminazene aceturate concomitantly with vaccines during vaccination campaigns for treatment of endemic trypanosomosis. This study showed that antibodies elicited by cattle vaccinated with the S19 vaccine persisted longer than those elicited by cattle simultaneously given the vaccine and the drug. Nicoletti *et al.* (1987) have reported similar findings on calves vaccinated with *B. abortus* S19 vaccine and supplemented with chlortetracycline.

It is concluded that Sudanese cattle under traditional farming system, where heifers reach maturity at about three years of age, can be vaccinated with full doses of S19 vaccine at 3-24 months of age, whereas adults with reduced ones. But 24-months-old calves and adult cattle should be marked upon vaccination to be recognized on

subsequent tests. Diminazene aceturate and other drugs that have brucidal effect should not be inoculated concomitantly with the vaccine.

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