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Frozen and fresh *Anaplasma centrale* vaccines in the protection of cattle against *Anaplasma marginale* infection*

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Les auteurs ont évalué l'immunité sur des bouvillons frisons âgés de 12 mois contre l'anaplasmose à *Anaplasma marginale* conférée par un vaccin réfrigéré d'une part, et un vaccin congelé d'autre part, contenant l'un et l'autre *A. centrale*. La parasitémie par *A. centrale* a été mesurée chez tous les sujets vaccinés. La plus grande chute de l'hématocrite a été 41,8 p. 100 pour le vaccin congelé et 40,3 p. 100 pour le vaccin réfrigéré. Tous les bouvillons ont guéri sans nécessité d'un traitement spécifique. Six mois après, les bovins des groupes vaccinés et ceux du lot témoin ont été éprouvés avec *A. marginale* à des doses allant de 10^6 à 10^7 ou 10^8. La parasitémie a varié de 1,2 à 4,0 p. 100 chez les vaccinés et de 10,3 à 12,0 p. 100 chez les témoins. La plus grande chute de l'hématocrite a été respectivement de 33,1 p. 100, 30,0 et 57,4 p. 100 dans les lots inoculés avec le vaccin congelé, le vaccin réfrigéré et ceux non vaccinés. Les bouvillons vaccinés ont guéri sans traitement spécifique, ce qui s'est révélé nécessaire chez 7 des 8 témoins. Les deux vaccins ont montré un degré similaire de protection contre *A. marginale* et ont évité une grave destruction érythrocitaire. *Mots clés* : Bovin - Anaplasmose - Vaccin vivant - *Anaplasma marginale* - *Anaplasma centrale* - Hématocrite - Immunité - Argentine.

INTRODUCTION

Shortly after his discovery of *Anaplasma centrale*, THEILER reported that considerable cross-immunity existed between this parasite and the more virulent *A. marginale* (15). As a result live *A. centrale* was adopted for routine vaccination in various anaplasmosis enzootic areas (5) while vaccines containing live or killed *A. marginale* were used in others.

Live vaccines for anaplasmosis consist of defibrinated blood from patent carriers of *A. centrale* or *A. marginale*. As these vaccines are generally chilled, their storage period is short (about one week), precluding thorough testing before use. This also hampers their application in areas remote from the producing center. To solve these disadvantages, various researchers have developed frozen live vaccines containing either *A. marginale* (17) or *A. centrale* (6, 7, 10, 12).

The immunity conferred by fresh *A. centrale* vaccine against *A. marginale* has been the subject of several investigations (1, 2, 4, 14, 18). The present report compares the protection conferred by fresh and frozen-stored *A. centrale* vaccines against challenge with *A. marginale*.

MATERIAL AND METHODS

Vaccines

Frozen *A. centrale* vaccine was prepared as reported recently (11). Briefly, *A. centrale*-infected blood from a donor calf was mixed with dimethyl sulfoxide (DMSO) and phosphate buffered saline (PBS) to yield a final concentration of 15 % DMSO and 10^9 *A. centrale* organisms per 2 ml of blood. Aliquots of 2 ml each were dispensed into plastic minicups, frozen at − 70 °C for 24 hours and then stored in liquid nitrogen. For use, the frozen pellets were thawed and each pellet of vaccine was diluted in 48 ml of 15 % DMSO in PBS. Each dose of 5 ml thawed vaccine contained 10^8 *A. centrale* organisms.

Fresh vaccine was obtained from a splenectomized calf with a 4 % *A. centrale* parasitemia on the day of bleeding. Blood was drawn into a flask containing 10 ml of 5 % sodium citrate for each 90 ml of blood. The number of parasites per ml was calculated from the red blood cell (rbc) count and from the percentage of infected rbc seen on a Giemsa stained thin blood film. The blood was diluted with PBS to yield a final concentration of 10^7 *A. centrale* in 5 ml of vaccine.

Challenge

An isolate of *A. marginale* without appendages « tails » obtained from an outbreak of anaplasmosis in the

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North of Argentina (Salta Province) was used for challenge. Blood from an infected animal was cryopreserved and, before use in the challenge experiments, it was inoculated into a splenectomized calf.

Experimental design

Twenty seven about 12-month old Friesian steers were obtained from and maintained in an anaplasmosis-free area. The steers were divided into 3 groups of 9 animals each. Groups A et B were vaccinated with frozen and fresh *A. centrale* vaccines, respectively. Group C was left as a non-vaccinated control. Response to vaccination was monitored by examining blood films, determining packed cell volume (PCV) and measuring body temperature. These examinations were performed on the day of vaccination and every other day for a period of 5 weeks starting 15 days after inoculation.

Six months later, each group subdivised into 3 subgroups of 3 steers each* and challenge doses of 10^6, 10^7 and 10^8 *A. marginale* organisms were administered to each subgroup, respectively. Blood films, PCV and body temperature were obtained again on the day of challenge and every second day for a period of 5 weeks starting 15 days after challenge.

Steers were treated (Oxytetracycline 20 mg/kg) when the PCV reached a lower level than 15 %.

RESULTS

Response of the steers to frozen and fresh *A. centrale* vaccine appears in table I. The mean prepatent period in steers inoculated with frozen vaccine was about 10 days longer than in the group inoculated with fresh vaccine. All animals showed *A. centrale* organisms in blood smears. The maximal parasitemia of each animal varied widely in both groups (from 0.5 to 12.0 %) but a higher mean parasitemia was observed in steers of group A. A considerable decrease of PCV was detected in most steers and similar mean values were observed in both groups. A slight increase in body temperature occurred in most of the cattle. All steers recovered without specific treatment.

* One non-vaccinated animal was discarded before the challenge for reasons unrelated to the present trial.

TABLE I Response of steers to frozen and fresh **A. centrale** *vaccines.*

Group	Type of vaccine	Average prepatent period (days)	Average maximal values		
			A. centrale parasitemia (%)	Decrease in PCV (%)	Temperature (°C)
A*	Frozen	39.8±3.95**	6.9±4.12	41.8±11.61	39.7±0.24
B*	Fresh	29.3±5.72	3.8±3.22	40.3±10.21	39.7±0.24

* *n = 9.*
** *Standard deviation.*

Response to *A. marginale* challenge is summarized in table II. Average prepatent periods varied from 21.7 to 31.0 days with no direct correlation to the number of organisms inoculated in each animal. Maximal *A. marginale* parasitemias varied from 1.2-4.0 % in vaccinated steers versus 10.3-12.0 % in non-vaccinated steers. Average maximal temperatures varied from 39.4 °C (group B) to 39.9 °C (group C).

The minimum PCV varied in individual vaccinated animals from 16 to 32 %. The average maximal decrease of PCV was 33.1 % in steers of group A, and 30.0 % in those of group B. All steers vaccinated with both types of vaccines recovered spontaneously from the *A. marginale* infection. The non-vaccinated steers showed a considerable red blood cell (RBC) destruction after the challenge resulting in an average maximal decrease of PCV of 57.4 % and 7 out of 8 animals required treatment with oxytetracycline.

DISCUSSION

As early as in 1944, *A. centrale* was successfully preserved for 254 days in solid carbon dioxide without added cryoprotectants (19). One ml of blood with a 3 % infection rate was sufficient to infect cattle. Later, 7 and 10 % glycerol were added before freezing (3, 13) to mitigate damage to the organisms during freezing and thawing.

Following the observation that DMSO protects mammalian erythrocytes from lysis during freezing-thawing (8) this reagent was applied in cryopreservation of intraerythrocytic organisms. Since penetration of glycerol in bovine rbc is considerably slower than in human rbc (9), DMSO appears to be preferable in freezing bovine intraerythrocytic organisms. However, the post-thawing viability of vaccines with *A. centrale*

cryopreserved with glycerol (7 ; MANGOLD, AGUIRRE, in preparation) seems to be longer than in those cryopreserved with DMSO (6).

Frozen *A. centrale* vaccines have been prepared using two different methods of conservation and application (10, 11). The doses of vaccine used in the present trial were similar to those recommended for routine field vaccination using fresh (16) and frozen (1) vaccines. The number of organisms per dose in the frozen vaccine was considerably higher than that in the fresh vaccine in order to compensate for possible lowered viability caused by the freezing-thawing process. It is likely that the longer prepatent period observed in this work with the frozen vaccine was due to such damage.

The response to both types of vaccine was a considerable decrease of PCV in most inoculated steers. These animals were about 1 year old when vaccinated and previous investigations have shown that anemia caused by *A. centrale* increase with the age of inoculated cattle (11). For this reason, in practice, the vaccine is recommended mainly for young animals.

The results of challenge with *A. marginale* showed that both vaccines conferred similar protection against the latter infection. In our experimental conditions different challenge doses triggered similar responses in the animals. The immunity induced by *A. centrale* was partial and did not prevent *A. marginale* infection resulting in erythrocyte destruction of various degrees. Nevertheless, this partial resistance allowed spontaneous recovery in all vaccinated cattle. On the other hand, the level of parasitemia and PCV decrease in non-vaccinated cattle showed that, without treatment, serious consequences from the *A. marginale* infection could be expected in 7 out of 8 steers. Other investigators (1, 2, 4, 14, 18) have also concluded that, despite immunological differences between *A. centrale* and *A. marginale*, immunization with *A. centrale* is indicated in cattle at risk because it reduces the virulence of subsequent infection with *A. marginale*.

The main advantage of the frozen over the fresh *A. centrale* vaccine is its longer storage period. This allows for thorough testing before the release to the market and permits transport and application in areas remote from the producing center.

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The immunity induced by frozen and fresh *Anaplasma centrale* vaccines against anaplasmosis caused by *A. marginale* was tested in 12-month old Friesian steers. *A. centrale* parasitaemia occurred in all cattle inoculated with both types of vaccine. The average maximal decrease in PCV for the frozen and fresh vaccines was 41.0 and 40.3 % respectively. All cattle recovered spontaneously. Vaccinated and control steers of the same age were challenged six months later with doses of 10^6, 10^7 or 10^8 *A. marginale* organims. Vaccinated cattle showed average maximal *A. marginale* parasitemia of 1.2-4.0 versus 10.3-12.0 % in control cattle. The average maximal decrease in packed cell volume (PCV) was 33.1 and 30.0 % for steers vaccinated with frozen or fresh vaccine, respectively, and 57.4 % for the non-vaccinated steers. All vaccinated cattle recovered spontaneously from the *A. marginale* infection while 7 out of 8 control steers required specific treatment. It thus appears that both frozen and fresh *A. centrale* vaccines are equally capable of inducing partial protection against infection with *A. marginale* and of preventing severe red blood cell destruction. *Key words* : Cattle - Anaplasmosis - Live vaccine - *Anaplasma marginale* - *Anaplasma centrale* - Packed cell volume - Immunity - Argentina.

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Se evaluó la inmunidad inducida por una vacuna refrigerada y otra congelada conteniendo *Anaplasma centrale* contra la anaplasmosis provocada por *Anaplasma marginale* en novillos Friesian de 12 meses de edad. Se comprobó la parasitemia por *A. centrale* en todos los bovinos vacunados. El descenso máximo del hematocrito fue de 41,8 p.100 (vacuna congelada) y de 40,3 p.100 (vacuna refrigerada). Todos los novillos se recobraron sin necesidad de tratamiento específico. Seis meses después se desafiaron los bovinos de los grupos vacunados y de los de un grupo testigo con dosis de 10^6, 10^7 o 10^8 *A. marginale*. La parasitemia fue de 1,2-4,0 p. 100 en los vacunados y de 10,3-12,0 p. 100 en los testigos. El descendo máximo del hematocrito fue de 33,1 p. 100, 30,0 p. 100 y 57,4 p. 100 en los inoculados con vacuna congelada, vacuna refrigerada y testigos, respectivamente. Los animales vacunados se recuperaron sin necesidad de tratamiento específico, en tanto que 7 de los 8 testigos lo necesitaron. Ambas vacunas mostraron igual nivel de protección contra *A. marginale* evitando una severa destrucción de los glóbulos rojos. *Palabras claves* - Bovino - Anaplasmosis - Vacuna viva - *Anaplasma centrale* - *Anaplasma marginale* - Hematocrito - Inmunidad - Argentina.

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