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RESEARCH COMMUNICATION
FREEZE-DRYING OF *ANAPLASMA MARGINALE*

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ABSTRACT

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Heparinized whole blood, heavily parasitized with *Anaplasma marginale*, was collected from 3 splenectomized oxen. Buffered lactose peptone (BLP) was added in equal volumes as a stabilizer and the mixture lyophilized in 2 mℓ aliquots after rapid freezing. The dried material was reconstituted with 2 mℓ sterile water and inoculated without delay. The product remained infective for at least 6 months when stored in an ordinary household deep-freeze unit.

Résumé

LA LYOPHILISATION DE L' ANAPLASMA MARGINALE

*Du sang entier héparinisé, fortement parasité par l'*Anaplasma marginale *a été récolté de 3 boeufs splénectomisés. Du peptone lactose ajusté à un pH donné (BLP) fut ajoué en volumes égaux comme stabilisateur et le mélange fut lyophilisé dans 2 mℓ d'aliquots après congélation rapide. Le matériel séché fut reconstitué avec 2 mℓ d'eau stérile et inoculé sans délai. Le produit demeura infectieux pour au moins 6 mois quand il était stocké dans un congélateur domestique ordinaire.*

INTRODUCTION

Attempts were made to lyophilize *Anaplasma*-infected bovine blood for possible replacement of the existing anaplasmosis blood vaccine issued by this institute. The present *Anaplasma centrale* vaccine as described by Potgieter (1979), has a limited shelf life and the infectivity has been found to be unsatisfactory. The potential advantages of a freeze-dried vaccine are therefore obvious. Ideally, such a vaccine should be infective via the subcutaneous route and should retain its infectivity at least at room temperature for the time required for dispatching and administering the vaccine. Storage and viability of such a vaccine would be a very important aspect from the producer's and farmer's point of view. The average farmer has facilities to keep a vaccine at 4 °C or even at −20 °C if necessary.

MATERIALS AND METHODS

Three batches of lyophilized *A. marginale* were prepared from different animals over a period of 9 months.

Experimental animals

The cattle used were of mixed European breeds, born and reared under tick-free conditions and splenectomized when 4–10 months old. Rectal temperatures and packed cell volumes (PCV) were determined daily during the primary *A. marginale* reactions. Giemsa-stained thin blood smears, prepared from the tip of the tail, were examined daily to quantify the rising parasitaemias and to establish the prepatent period of the *A. marginale* infections.

A. marginale *strain*

Only one strain, namely, the BW strain of *A. marginale* (Potgieter, Sutherland & Biggs, 1981), was used in this investigation. A blood stabilate kept at −196 °C was used to infect 3 donor oxen intravenously.

Preparation of buffered lactose peptone (BLP)

Dissolve 605,3 g of $Na_2HPO_4.12H_2O$ and 34,7 g KH_2PO_4 in 40 ℓ of distilled water. Heat to approximately 90 °C. Add 4 000 g of lactose and 800 g of proteose peptone.* Sterilize the solution by filtrating it through Seitz EK-SI discs.

Collection of infected blood

Blood was collected aseptically in 10 mℓ venoject tubes, containing sodium heparin as anticoagulant, from the jugular veins of the reacting animals (Table 1) and immediately refrigerated for cooling down to approximately 4 °C. The blood was then pooled and mixed with an equal volume of BLP, also at 4 °C. BLP is routinely made up as a stock solution and is generally applied in vaccine production at this institute. It consists of a phosphate buffer, 10% lactose and 2% proteose peptone, and has a pH of 7,5.

TABLE 1 Collection of *A. marginale* infected blood

Batch	Animal No.	Parasitaemia %	PCV %
1	2950	80	24
2	8625	60	25
3	3095	86	15

Rapid freezing of blood-BLP mixture

Two mℓ aliquots of the blood BLP mixture was transferred into standard 5 mℓ glass vials (vaccine bottles), sealed with rubber stoppers of the split type and placed in an upright position in a dry ice-ethanol bath approximately 1,5 cm deep. Temperatures in the dry ice-ethanol mixture reached −65 °C. The material in the vials took ±40 s to reach 0 °C and <3 min to reach −60 °C. Twelve min were spent in transferring the 50 vials manually and getting the material frozen as indicated above.

The vials were then taken from the bath and dried with a cloth. The stoppers were only loosely inserted and left in that position to allow the moisture to escape during the drying process before the vials were placed on the pre-cooled tray (−30 °C) of the freeze-drying machine. After they were removed from the alcohol, the temperature of the material increased to an average of −15°C before the tray was loaded into the machine.

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Freeze-drying cycle

The first batch was processed together with other vaccines in an Edwards freeze-drying machine with a 20 ℓ capacity which carried only a 6 ℓ load at the time. Batches 2 and 3 were dried in a modified, locally constructed machine with an 80 ℓ capacity, carrying 10 ℓ.

The condenser temperature of both machines was −60 °C, while the shelf temperatures ranged from −30 °C to 35 °C during the average 17 h drying cycle. At the end of the freeze-drying cycle the vacuum was 10 Pa.

A pneumatic stoppering system was used to seal the vials in the shelves under vacuum. With the stoppers fully seated the vials were removed and the stoppers fixed with metal caps. The time taken for this was approximately 10 min. The residual moisture content of the material was not determined.

Storage

All the vials were immediately placed in an ordinary household deep-freeze unit at approximately −20 °C.

Inoculation

Each vial was rehydrated with 2 mℓ of sterile water (4 °C) and the contents injected immediately (Table 2). Approximately 15 min elapsed between the removal of the vials from cold storage and the injection of the rehydrated material.

Results

The results of these trials are summarized in Table 2. It was found that the lyophilized blood-BLP mixture was infective when administered both intravenously and intramuscularly after rehydration. One attempt to infect an animal subcutaneously failed.

So far the material from the 1st batch has retained its infectivity for 6 months when stored, as indicated in Table 2. However, if left at 4 °C in a refrigerator after it had been kept for 10 weeks at temperatures of approximately −20 °C, it apparently loses its infectivity (Table 2).

TABLE 2 Summary of trials with 3 different batches of lyophilized *A. marginale*

Batch	Animal No.	Storage		Route of 2 mℓ inoculum	Pre-patent period of *A. marginale* infection (days)
		Temp.	Days		
1	2378		3	i/v	27
	9416		3	i/v	23
	2928		3	s/c	—
	1643	−20 °C	84	i/m	30
	1403		84	i/v	26
	3406		179	i/v	29
	3350		7	i/v	—
	3380	4 °C*	7	i/v	—
	3142		7	i/m	—
	3406		7	i/m	—
2	3953	−20 °C	4	i/v	24
3	2895	−20 °C	2	i/v	28

* Stored at −20 °C for 10 weeks before being transferred to 4 °C

i/v=intravenous, s/c=subcutaneous, i/m=intramuscular

Discussion

The fact that *A. marginale* was successfully lyophilized 3 times by this uncomplicated technique is very promising. At this stage, however, 2 aspects of the results are disappointing: the inability of the material to infect an ox via the subcutaneous route and the loss of infectivity if kept at elevated temperatures for a reasonable period. Both these factors require further investigation because they limit the practical application of this technique in possible future vaccine production.

References

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