Onderstepoort J. vet. Res., 55, 125–126 (1988)

RESEARCH COMMUNICATION

FREEZE-DRYING OF *AEGYPTIANELLA PULLORUM*

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ABSTRACT

BARTKOWIAK, R. A., HUCHZERMEYER, F. W., POTGIETER, F. T., VAN RENSBURG, L., LABUSCHAGNE, F. J. & VAN BILJON, B. J., 1988. Freeze-drying of *Aegyptianella pullorum*. *Onderstepoort Journal of Veterinary Research*, 55, 125–126 (1988)

Heparinized whole blood, parasitized with *Aegyptianella pullorum*, was collected from 2 fowls. Buffered lactose peptone (BLP) was added v/v as a stabilizer and the mixture lyophilized in 2 mℓ aliquots after rapid or slow freezing. At different stages during the freeze-drying process, as well as after lyophilization and reconstitution with 1,8 mℓ of sterile water, samples were taken and injected into pullets. Infectivity was maintained throughout. However, the prepatent period was lengthened after freezing and particularly after lyophilization when there was some loss of viability.

INTRODUCTION

Cryopreservation of *Aegyptianella pullorum* in liquid nitrogen, using citrated blood without any cryopreservative was first reported by Huchzermeyer (1965). Raether & Seidenath (1977) used 10 % glycerin as cryoprotectant when freezing *A. pullorum* containing heparinized blood in liquid nitrogen. They also added further heparin to the sample on thawing out.

The successful lyophilization of *Anaplasma marginale* by Potgieter & Bester (1981) opened new approaches for research on anaplasmosis vaccine. For vaccination purposes, it is important to obtain freeze-dried material capable of retaining infectivity, even at room temperature.

Because of the high cost of experimentation with a bovine *Anaplasma* sp., it was decided to use *A. pullorum* in young chickens as a model. This paper reports the results of an attempt to freeze-dry *A. pullorum* in chicken blood.

MATERIALS AND METHODS

Experimental animals

Twelve seven-week old white Leghorn pullets were used in the 6 different groups. The 2 donor birds were of the same age, breed and sex.

Aegyptianella pullorum strain

The *A. pullorum* strain A61/86 was isolated from backyard fowls in the vicinity of Pretoria in January 1986. The infected blood used for this experiment was in its 10th intravenous bird to bird passage. Final parasitaemia of the mixed blood was 130 parasites per 100 microscopic fields of view (approximately 100 erythrocytes per field of view).

Buffered lactose peptone (BLP)

The preparation of BLP was carried out as described by Potgieter & Bester (1981).

Collection of infected blood

The infected blood was collected from the severed jugular veins of 2 infected fowls into a plastic beaker containing 1000 i.u. of sodium heparin as anticoagulant. Ninety mℓ of pooled blood thus obtained was immediately placed on a cooling tray and cooled to 4 °C, after which an equal volume of BLP, also at 4 °C, was added.

Freezing of the blood-BLP mixture

Two mℓ aliquots of the blood-BLP mixture were transferred into standard 5 mℓ glass vials (vaccine bottles) and sealed with rubber stoppers of the split type.

Received 1 December 1987—Editor.

Half the vials (Group A) were placed upright into the vapour phase of a liquid nitrogen container for rapid freezing and left for 30 min, while the other half of the samples (Group B, intended for slow freezing in the freeze-drying machine) were left at 4 °C on the cooling tray.

Freeze-drying cycle

The samples were freeze-dried in an Edwards freeze-drying machine with a 20 ℓ capacity, which carried only a 6 ℓ load at the time. The stoppers of both groups were inserted in the correct position for freeze-drying under laminar flow. The following drying cycle was used:

- — Precool shelves to a temperature of −30 °C and the condenser to −60 °C
- — Samples placed on the shelves allowed to freeze for 3 h to reach −30 °C
- — Butterfly valve opened to chamber vacuum 10 Pa
- — Temperature set to −10 °C and samples dried for 42 h
- — Temperature set to +10 °C and samples dried for 7 h
- — Samples sealed off under vacuum in the machine with the pneumatic stoppering system
- — Metal caps fastened onto bottles with the capping machine, while still maintaining +10 °C.

Infectivity tests

At different stages in the freeze-drying cycles, samples were taken and injected intravenously each time into 2 pullets. The following samples were tested: fresh heparinized blood; blood-BLP mixture (referred to as blood below) before freezing; blood after rapid freezing (A) and rapid thawing; rapid frozen lyophilized blood reconstituted; blood after slow freezing (B) and rapid thawing; and slow frozen lyophilized blood reconstituted.

One mℓ of the 1st sample was injected into each of the 2 birds. Because of the dilution with BLP, the dosage of all the following samples was 2 mℓ. The lyophilized samples were reconstituted with 1,8 mℓ of sterile distilled water at room temperature 30 min after the end of the freeze-drying process and inoculated within 10 min of reconstitution.

Monitoring of the parasitaemia

From the 4th day after inoculation of the fresh and frozen samples and from the 2nd day of the freeze dried samples, blood smears were prepared daily, except at weekends, from all the birds. The smears were then fixed with May-Grünwald Giemsa for 1 min and stained with Giemsa's stain in buffered distilled water (pH 6,5) for 30 min at 3 %. A maximum of 25 fields of view

TABLE 1 Infectivity of lyophilized *Aegyptianella pullorum* after i.v. infection of 2 pullets per treatment

Treatment	Fresh heparinized blood					
		Chilled, BLP added				
			Rapid freezing		Slow freezing	
				Lyophilization		Lyophilization
Parasitaemia						
Prepatency: days p.i.	3	3	5–6	17	6	16–19
Peak: days p.i.	6	5–6	8–9	20–21	9–12	18–20
Mean No. of parasites/10 000 rbc's at peak	2 992	2 668	850	1 360	364	650

p.i. = post inoculation

under oil immersion was examined and parasite counts expressed as parasites per 100 fields of view (± 10 000 red blood cells), were done. The mean number of parasites per 10 000 red blood cells was calculated at peak parasitaemia.

RESULTS

The results of the infectivity trials are summarized in Table 1.

It was found that *A. pullorum* blood taken from all investigated stages of the freeze-drying process was infective when administered intravenously.

In comparison with the 3 days of fresh blood, the prepatent period of frozen blood was found to be extended to 6 days, and to approximately 17 days after lyophilization. In all cases the peak of parasitaemia occurred 2–6 days after the appearance of the first parasites. These results were obtained in spite of the relatively low parasitaemia in the original material, which was due to difficulties experienced in the timing of the trial.

DISCUSSION

The fact that infectivity was retained through all treatments indicates that *A. pullorum* could further be used as a model in *Anaplasma* vaccine research. Since the length of the prepatent period is thought to be dose dependent (Huchzermeyer, 1965), it appears that freezing, to a lesser degree, and the subsequent drying and reconstitution, to a higher degree, reduce the number of viable organisms in the blood.

From the delay of 5–7 days in reaching peak parasitaemia after freezing unprotected blood, reported by Huchzermeyer (1965), compared with that of the results (3–5 days) obtained in this trial, it appears that BLP could possibly in itself have some cryoprotective action on chicken blood infected with *Aegyptianella*. This aspect might warrant further investigation.

The peak levels of parasitaemia did not show a consistent pattern. While it appears that parasitaemia was lowest after slow freezing, this could very well be a function of individual host response, with some birds being able to suppress the multiplication of *Aegyptianella* earlier or more successfully than others.

REFERENCES

HUCHZERMEYER, F. W., 1965. Das Tiefgefrieren von *Aegyptianella pullorum* in flüssigem Stickstoff mit einigen Bemerkungen über die künstliche Infektion beim Huhn. *Berliner und Münchener Tierärztliche Wochenschrift*, 78, 433–435.

POTGIETER, F. T. & BESTER, J. B., 1981. Freeze-drying of *Anaplasma marginale*. *Onderstepoort Journal of Veterinary Research*, 48, 179–180.

RAETHER, W. & SEIDENATH, H., 1977. Survival of *Aegyptianella pullorum*, *Anaplasma marginale* and various parasitic protozoa following prolonged storage in liquid nitrogen. *Zeitschrift für Parasitenkunde*, 53, 41–46.

Printed by the Government Printer, Private Bag X85, Pretoria, 0001