

RESEARCH COMMUNICATION

Cryopreservation of sheathed third-stage larvae of *Nematodirus spathiger*

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ABSTRACT

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A technique for the cryopreservation of sheathed third-stage larvae of *Nematodirus spathiger* is described. It consists of incubating larvae in 5% (v/v) ethylene glycol for 20 min at room temperature, followed by 1 min of incubation in 40% (v/v) ethylene glycol at 0°C. The average recovery of adult worms was 28,5% for two adult sheep infected with these larvae.

Keywords: Cryopreservation, sheathed third-stage larvae, *Nematodirus spathiger*

Previously, cryopreserved exsheathed larvae of *Nematodirus spathiger* larvae showed poor infectivity for adult sheep (Van Wyk, Gerber & Alves 1984).

The present study was conducted in order to improve larval infectivity by using a two-step incubation procedure during cryopreservation.

Sheathed *N. spathiger* larvae were preincubated in 5% ethylene glycol dissolved in an 0,09% saline solution at room temperature for 20 min. Subsequently, 0,6-ml samples of this material were transferred to cryotubes already containing 0,35 ml of ethylene glycol (99,9% v/v), in order to obtain a final concentration of 40% cryoprotectant. After incubation for 1 min at 0°C in an ice bath, the samples were plunged into liquid nitrogen.

After 30 d of storage, larvae were thawed and washed twice in 10 ml of tap water at 40°C and their survival was assessed by motility. Judged by this criterion, the survival rate exceeded 96%.

Two 12-month-old, male Dorper sheep were each infected *per os* with 10 000 motile, sheathed, cryopreserved third-stage larvae. Faecal worm-egg counts of 300 and 600 eggs/g were recorded for these sheep after a normal prepatent period of 21 d. At necropsy 30 d after infection, 26 and 31% of the larvae administered were recovered as adult worms. In the experiment conducted by Van Wyk *et al.* (1984) only 14,5% of the larvae dosed were recovered as adult worms.

This study demonstrates that the cryoprotection of infective larvae of *N. spathiger* was improved after a two-step incubation in cryoprotectant.

REFERENCES

- VAN WYK, J.A., GERBER, H.M. & ALVES, REGINA M.R. 1984. Methods of infesting sheep with gastro-intestinal nematodes after cryopreservation: dosing of larvae in gelatin capsules compared to dosing of larvae in water suspension. *Onderstepoort Journal of Veterinary Research*, 51:217-221.