

RESEARCH COMMUNICATION

An improved technique for the cryopreservation of *Gaigeria pachyscelis* (Sandveld hookworm)

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ABSTRACT

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A technique for the cryopreservation of third-stage larvae of *Gaigeria pachyscelis* is described. It consists of incubating sheathed infective larvae at 37°C in 40% (v/v) ethylene glycol for 7 min, followed by 2 min at 0°C in 82,8% (v/v) cryoprotectant mixture, prior to transferring the larvae to liquid nitrogen. The survival rate obtained with this technique is consistently high: 69,1% as assessed by motility.

Keywords: Cryopreservation, sheathed third-stage larvae, *Gaigeria pachyscelis*, Sandveld hookworm

Recently Tittoy & Schwan (1995), using a high concentration of ethylene glycol, successfully cryopreserved sheathed third-stage larvae of *Gaigeria pachyscelis* (Sandveld hookworm). The present study was conducted in order to improve the larval survival rate of stored *G. pachyscelis* larvae by preincubating sheathed third-stage larvae in 40% (v/v) ethylene glycol dissolved in 0,9% saline for 7 min at 37°C. Volumes of 0,24 ml were transferred to cryotubes containing 0,6 ml cryoprotectant mixture (30% ethylene glycol plus 70% DMSO) to give a final concentration of 82,8% (v/v) cryoprotectant (32,8% ethylene glycol, 50% DMSO, 17,1% saline). After incubation for 2 min at 0°C, each sample was plunged into liquid nitrogen in which it was also stored.

After 30 d, larvae were thawed, washed twice in tap water at 40°C and examined for motility. Judged by this criterium, the survival rate was 69,1%.

A 3-month-old male Dorper sheep was infected *per cutem* in the groin region (Reinecke 1973) with 700 motile cryopreserved third-stage larvae. A faecal egg count of 400 eggs/g was recorded in this sheep after a normal prepatent period of 78 d. At necropsy on day 80, 49 adult worms were recovered.

This study demonstrates that the addition of cryoprotectants in two steps to sheathed *G. pachyscelis* third-stage larvae followed by rapid cooling, increased their level of survival.

REFERENCES

- REINECKE, R.K. 1973. *The larval anthelmintic test in ruminants*. [Pretoria]: Department of Agricultural Technical Services (Technical Communication, no. 106).
- TITTOY, G.A.P. & SCHWAN, E.V. 1995. Cryopreservation of sheathed third-stage larvae of *Gaigeria pachyscelis* (Sandveld hookworm). *Onderstepoort Journal of Veterinary Research*, 62: 63–64.