

RESEARCH COMMUNICATION

Cryopreservation of sheathed third-stage larvae 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 third-stage larvae in 80% (v/v) ethylene glycol as a cryoprotectant for 30 s at 0 °C, prior to transfer into liquid nitrogen. The survival rate, as assessed by motility, was 37,6% after 30 d cryopreservation. A sheep infected percutaneously with 700 live cryopreserved third-stage larvae, harboured 41 adult worms (infectivity rate: 5,8%) when necropsied 78 d later.

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

Although there are several reports on the successful cryopreservation of exsheathed third-stage larvae of ovine and bovine nematodes (Van Wyk, Gerber & Van Aardt 1977; Campbell, Blair & Ergerton 1973), there is no information on the cryopreservation of sheathed third-stage larvae of *Gaigeria pachyscelis* (Sandveld hookworm).

Third-stage larvae of *G. pachyscelis* were harvested from faecal cultures after 5 d of incubation (Whitlock 1956).

The two parameters evaluated were:

- Sheathed third-stage larvae's tolerance of different concentrations of the cryoprotectant ethylene glycol (Merck, Darmstadt, Germany) dissolved in 0,09% saline at a constant incubation time of 2 min at 0 °C and at 37 °C.

- Survival rates (%) of sheathed third-stage larvae after various incubation times in three concentrations of ethylene glycol, followed by 30 d of cryopreservation in liquid nitrogen.

At a constant incubation time of 2 min, motility of the larvae declined with increasing concentrations of cryoprotectant. Motility was better after incubation in 80% ethylene glycol at 0 °C than at 37 °C (Fig. 1). The highest survival rate of 37,6% was obtained when larvae were incubated in 80% ethylene glycol for 30 s at 0 °C, prior to cryopreservation (Fig. 2). After 30 d of cryopreservation larvae were thawed and washed twice in tap water at 40 °C. The larvae were examined for motility and 700 motile larvae were used to infect a 4-month-old Dorper ewe-lamb percutaneously in the groin region (Reinecke 1973).

Eggs of *G. pachyscelis* were recovered from the faeces of the animal 78 d after infection and a faecal egg count of 400 eggs/g was recorded. At necropsy on the

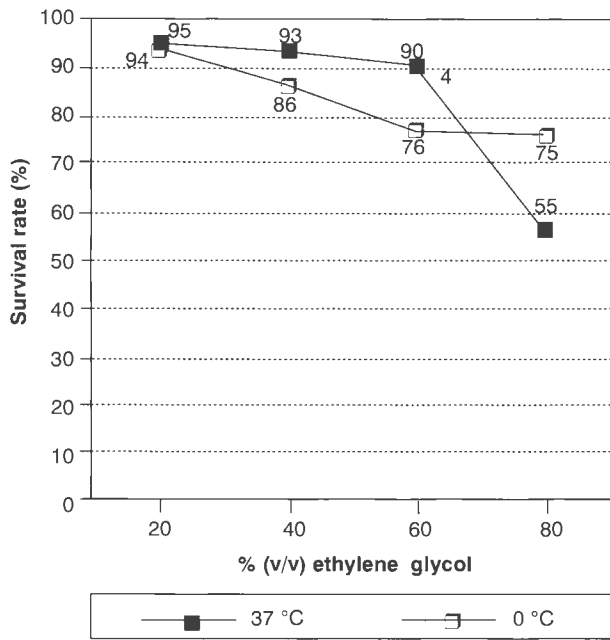


FIG. 1 Survival rate (%) of *Gaigeria pachyscelis* third-stage larvae after 2 min of incubation at 0 °C or 37 °C in various concentrations of ethylene glycol

same day, 41 adult worms were recovered, reflecting an infectivity rate of 5,8%.

This study has demonstrated for the first time that sheathed third-stage larvae of *G. pachyscelis* can be cryopreserved and that they retain their viability after thawing.

REFERENCES

CAMPBELL, W.C., BLAIR, L.S. & ERGERTON, J.R. 1973. Unpaired infectivity of the nematode *Haemonchus contortus* after

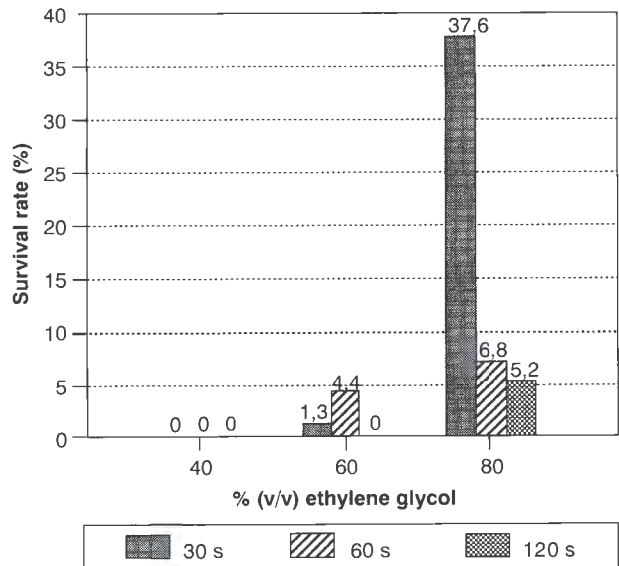


FIG. 2 Survival rate (%) of *Gaigeria pachyscelis* third-stage larvae after incubation in three concentrations of ethylene glycol for various lengths of time and subsequent cryopreservation for 30 d

freezing for 44 weeks in the presence of liquid nitrogen. *Journal of Parasitology*, 59:425–427.

REINECKE, R.K. 1973. *The larval anthelmintic test in ruminants*. [Pretoria]: Department of Agricultural Technical Services. (Technical Communication; no 106.

VAN WYK, J.A., GERBER, H.M. & VAN AARDT, W.P. 1977. Cryopreservation of the infective larvae of the common nematodes of ruminants. *Onderstepoort Journal of Veterinary Research*, 44: 173–194.

WHITLOCK, H.V. 1956. An improved method for the culture of nematode larvae in sheep faeces. *Australian Veterinary Journal*, 32:141–143.