ABSTRACT


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-mt 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