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CRYOPRESERVATION OF INFECTIVE LARVAE OF TRICHOSTRONGYLUS VITRINUS[1]

Metin Alabay[2] **Zişan Emre**[3] **Harun Çerçi**[4]

Trichostrongylus vitrinus enfektif larvalarının dondurularak saklanması

Özet: *Kılıfları çıkarılmış 3. dönem Trichostrongylus vitrinus larvaları fizyolojik su içinde sıvı azotta dondurulmuş ve 249 gün saklanmıştır. Çözüldükten sonra canlı kalan larva oranı % 29.2 olmuştur. Daha sonra larvalar, dondurmanın enfektivite üzerindeki etkisini saptamak için 5 koyuna oral olarak verilmiştir. Larvaların enfektivitesi zayıf bulunmuştur.*

Summary: *Exsheathed third stage larvae of Trichostrongylus vitrinus suspended in physiological saline were frozen in liquid nitrogen and then stored for 249 days. After thawing, the percentage of surviving larvae was found to be 29.2 %. Larvae were then orally transmitted to 5 sheep to determine the effect of freezing on infectivity. The infectivity of these larvae was poor.*

Introduction

Successful cryopreservation of helminths comprising several different cell types with differing volumes and permiability characteristics is intrinsically difficult to achieve. Those helminth species that can be cryopreserved are the free-living larvae of animal nematodes (8). The infective larvae of many of these species can be successfully cryopreserved simply by suspension in tap water and slow coling (1°C / min). Survival levels, as with Haemochus (3), are often extremely good. Those nematode species whose free-living third stage

1 This study was supported by Turkish Atomic Energy Agency, Ankara.

2 Assoc. Prof. Lalahan Nuclear Reseaıch Institute of Animal Health, Parasitology Laboratory, Lalahan, Ankara.

3 DVM, PhD. Lalahan Nuclear Research Institute of Animal Health, Parasitology Laboratory, Lalahan, Ankara.

4 BSc. Lalahan Nuclear Research Institute of Animal Health, Parasitology Laboratory, Lalahan, Ankara.

larvae retain the cuticle of the second stage as a protective sheath, invariably have to be exsheathed before they can be successfully cryopreserved (2, 4, 9, 10, 11). Artificial exsheathment is likely to function by removing a barrier to water movement allowing the larvae to dehydrate during cooling (8).

Nematodes which remain enterily within a host throughout their life cycles, together with Schistosoma and Taenia, all appear to require the incorporation of crypoprotective additives (7, 8). Conversely, for most of the free-living larvae of nematodes of domestic animals, cryoprotectant addition leads to reduced survival (5, 9).

Several benefits could be gained from long term storage of cryopreserved nematode larvae. The considerable expenditure of time, labor and funds to continually maintain monospecific isolates in animals would be reduced and the risk of accidental contamination would be minimized.

Since Parfitt (11) reported that larvae of *Nematodirus battus* survive freezing in liquid nitrogen and exsheathed *Haemonchus contortus* remain infective after four weeks cryopreservation (2), there has been increased interest in deep freeze storage of nematode larvae. *Ancylostoma ceylanicum* (16), *A. caninum* (11), *Dictyocaulus viviparus* (9), *Schistosoma mansoni* (7), *Nippostrongylus brasiliensis* (10), *Cooperia oncophora*, *Haemonchus contortus*, *Nematodirus spathiger*, *Trichostrongylus sp.*, *Oesophagostomum sp.* and *Ostertagia sp.* (4, 5, 6,, 13) have all been successfully cropreserved. Van Wyk et al. (14) examined the viability and the infectivity of 19 ruminant species after freezing and found that generally all were infective after this treatment. Campbell et al. (3) also reported that frozen *H. contortus* were as infective as normal larvae after 44 weeks of storage.

The present study on cryopreservation of third stage larvae of *T. vitrinus* was made to investigate the viability and infectivity of these larvae.

Materials and Methods

Infective larvae of *Trichostrongylus vitrinus* were cultured from faeces passed by a sheep with a monospecific infection. The method of obtaining clean infective larvae of Trichostrongylus has been described before (1). Prior to cryopreservation, the larvae had been sto-

red in tap water in a refrigerator for about 6 weeks. Larvae were exsheathed with 0.16 % NaOCl. As soon as mass exsheathment had started as viewed under dark background illumination (approx. after 45 min.), NaOCl was removed by centrifugation and the larvae resuspended in 0.09 % NaCl solution. Then, larvae were placed in five eppendorf tubes each containing 15000 larvae in 1 ml. 0.09 % NaCl solution. The tubes were frozen by placing them directly into liquid nitrogen.

Frozen larvae were stored for 249 days to determine their survival capability. Larvae were thawed quickly by placing the tubes into a water bath at 37°C. immediately upon removing them from storage in the liquid nitrogen. After the larvae were thawed, the percentage of live larvae was determined on the basis of motility. Five doses containing approximately 4500 larvae were then prepared. These larvae were then orally given to five sheep to determine the effect of freezing on infectivity. Faceal agg counts were carried out using the McMaster method 14 days after inoculation of larvae.

Results

The percentages of *T. vitrinus* larvae surviving storage for 249 days in liquid nitrogen were 31.3, 34.4, 33.3, 23.9 and 23.1 % (a mean of 29.2 %) in the five samples. Larvae that survived freezing and thawing were not as motile as nonfrozen larvae.

It this study, because it was not possible to slaughter the sheep to make worm counts, the infectivity of larvae could only be assessed by faecal egg counts. Although all sheep became infected with frozen larvae, faecal egg counts were very low. Egg counts reached maximum values of 50 to 150 egg per gramme of faeces on day 38th of the infection.

Disscussion

At the present study, larvae of *T. vitrinus* were frozen by placing them directly into liquid nitrogen. That means rapid cooling has been used and 29.2 % survival of larvae obtained. Coles et al. (5) used both rapid and slow cooling for cryopreservation of exsheathed *T. colubriformis* and *T. axei* larvae and obtained 35 and 0 % survival with rapid and 80 and 95 % with slow cooling rates respectively. They also reported that oral challenge with frozen larvae was slightly success-

ful with intestinal nematodes and laparotmy was needed to reestablish a successful culture of *T. colubriformis*. Isenstein and Herlich (6) stored ensheathed *T. axei* and *T. collubriformis* infective larvae in liquid nitrogen vapour by cooling at a rate of 1°C / min. and found low percentage of larvae surviving cryopreservation. However, larvae that survived were as infective as nonfrozen larvae in rabbits. These results show better recovery of larvae and infection could be obtained after slow cooling of exsheated larvae and following laparotomy.

Nevertheless, Campbell and Thomson (4) cropreserved exsheathed larvae of *T. colubriformis* by rapid cooling and obtained 71–93 % survival. Moreover, Van Wyk et al. (14, 15) reported that *T. colubriformis*, *T. axei* and *T. falculatus* were viable after 2 years of cryopreservation by rapid cooling, a mean of more then 90 % of the L_3 being alive when thawed after this period. They also found that sufficient numbers of cryopreserved L_3 of *T. falculatus* and *T. colubriformis* developed when dosed per os in suspension without having to resort laparotomy.

Results reported in the present experiment demonstrated that cryopreserved larvae of *T. vitrinus* were neither as viable nor infective as reported for other *Trichostrongylus sp.* (15). However, as this study was of limited scope, further more extensive experiments need to be completed to confirm these findings.

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