roditic duct. Cirrus sac 0.5–0.67 long containing elongated seminal vesicle, prostatic complex, and cirrus. Hermaphroditic duct long, measuring 0.4–0.6 in length, extending intercecally from genital pore.

Ovary intercecal, in posterior ⅔ of body, 0.11–0.13 by 0.10–0.12. Vitelline follicles relatively large, completely surrounding body ventrally and dorsally from posterior tip of cirrus sac to posterior end of body, interrupted at level of ovary and each testis. Uterus extending intercecally from ovary differentiating into metraterm near junction with hermaphroditic duct. Receptaculum seminis uterinum present. Eggs relatively few, large 0.07–0.12 by 0.06–0.08. Excretory vesicle Y-shaped.

Remarks

Tormopsolus spatulatum is close to T. filiformis as far as long prepharynx, long forebody, vitellaria interrupted at level of ovary and each testis, and posterior extent of cirrus sac but differs from it by possessing a spatulate prepharyngeal region and very long esophagus. T. spatulatum is separated from T. osculatus and T. orientalis in having a much longer forebody (proportionately twice as long), longer prepharynx, spatulate pharyngeal region, and very long esophagus; in T. orientalis the esophagus is absent, and in T. osculatus it is very small. The vitelline follicles are interrupted at the level of each testis and ovary in T. orientalis as in the new species. In T. lintoni the ovary is not separated from the anterior testis by a band of vitellaria and the prepharyngeal region is not spatulate in addition to other morphological differences.

The specific name spatulatum refers to the shape of prepharyngeal region.

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Cryopreservation of Infective Third-Stage Larvae of Trichostrongylus axei and T. colubriformis

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ABSTRACT: Trichostrongylus axei and T. colubriformis infective third-stage larvae were frozen and stored for varying periods at -170 C: 10 to 80 days for T. axei and 10 to 38 days for T. colubriformis. Thawed, surviving larvae were used to infect rabbits. Survival percentages changed little between worms stored for the period of the test. Larvae surviving freezing, storage, and thawing were as infective as non-frozen larvae in rabbits.

Several benefits could be gained from long-term storage of nematode infective larvae. The considerable expenditure of time, labor, and funds to continually maintain monospecific isolates in culture animals would be reduced and the risk of accidental contamination would be minimized. Moreover, many monospecific isolates with particular genetic characteristics could be set aside for future study.

Various accounts concerning the ability of nematodes to survive the effects of subzero temperatures date back as far as Spallanzini (1776). Since then, sufficient evidence from various workers (Weinman and McAllister, 1947; Anderson and Levine, 1968; Muller, 1970) has accumulated to indicate that cryopreservation of nematodes is feasible and could be an important and useful laboratory procedure.

Therefore, the following study on cryo-
preservation of infective third-stage larvae was made with particular interest directed at the subsequent infectivity of these larvae.

**Materials and Methods**

Infective third-stage larvae of *Trichostrongylus axei* and *T. colubriformis* were cultured and isolated from feces passed by lambs with monospecific infections. Prior to cryopreservation, the larvae had been stored in tap water in a refrigerator (approximately 4 C) for several weeks. Subsequently, they were divided into six batches of *T. axei* and three batches of *T. colubriformis* with each batch usually containing approximately 2.5 million larvae in distilled water. The larvae in each batch were concentrated on a 5.5-cm-diameter filter paper disc, using a Buchner funnel at low vacuum. Each moist filter paper disc was then placed against the inner wall of a 13-dram plastic snap cap vial. The vials were cooled to -80 C at an approximate rate of 1 C per minute in a liquid nitrogen controlled-rate freezer* and stored in liquid nitrogen vapor (approximately -170 C).

About the same time the larvae were frozen, five rabbits each were inoculated with 5,000 *T. axei* and 5,000 *T. colubriformis* infective larvae that had been stored at 4 C, but not frozen, to provide a baseline infection.

Frozen larvae were stored for different time intervals to determine survival capability, and inoculated into rabbits to determine infectivity. Larvae were thawed quickly by pouring room temperature distilled water into the vials immediately upon removing them from storage in the liquid nitrogen vapor.

After the larvae were thawed, the percentage of live larvae was determined on the basis of motility, and doses containing approximately 5,000 larvae were prepared. Three lots of five rabbits each were inoculated with an aggregate dose of 5,000 *T. axei* and 5,000 *T. colubriformis* larvae stored 10, 24, and 38 days, respectively. Three lots of five rabbits each were inoculated with 5,000 *T. axei* larvae stored 45, 67, and 80 days, respectively.

All rabbits were killed 25 days after inoculation and examined for parasitic nematodes. Stomachs of rabbits inoculated with *T. axei* and small intestines of those with *T. colubriformis* were placed in pepsin–HCl digestion fluid for 6 hr at 40 C. Worm counts were based on the number of helminths found in duplicate 10% aliquots.

**Results and Discussion**

The percentage of larvae surviving storage for 10 days was 6.18 for *T. axei* and 7.60 for *T. colubriformis*. Low-temperature storage for periods longer than 10 days apparently had little effect on the percentage of survivors (Table 1). We assume that the stresses of freezing and thawing were responsible for most of the larval deaths and that much of the death loss in our experiments was attributable to mechanical damage by freezing fluid external to the worms. The work of Andersen and Levine (1968), who reported 50 per cent survival of *T. colubriformis* larvae after 128 days at -95 C following desiccation, supports this assumption.

Larvae that survived freezing and thawing were as infective as nonfrozen larvae in rabbits.

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* Canaleo, Inc., Rockville, Md. “Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable."
(Table 1). This infectivity indicates that cryopreservation may be a useful technique in nematode parasitology. Since most parasitic nematodes have a high level of fecundity, the high mortality, which probably can be reduced, appears to be a surmountable problem.

Literature Cited


Freshwater Larval Trematodes. XXIX. Life Cycle of Guaicaipuria parapseudoconcilia sp. n.¹

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Abstract: The cercaria of Guaicaipuria parapseudoconcilia sp. n. parasitizes the freshwater snail, Pomacea glauca, and encysts in the gills of the tadpoles, Engystomops pustulosus. Cysts, 13 days old, when fed to a domestic duckling developed into the adults in the bursa Fabricii, with the expulsion of eggs 12 days after the feeding. Miracidia penetrated one of the laboratory-raised snails, and cercariae emerged 41 days postinfection. These cercariae proved identical with those from field material. G. parapseudoconcilia, on the basis of adult characters, is indistinguishable from G. pseudoconcilia, the only other species in the genus, but is an independent entity when the cercarial characters are considered: in the former species the digestive tract is absent beyond the pharynx, while in the latter the esophagus is well developed and the intestinal ceca extend to the posterior end of the body; moreover, the former employs tadpoles as the second intermediate host in relation to the freshwater fish, Rivulus hartii (Boelenger), in the latter.

The genus Guaicaipuria Nasir, Díaz, and Marcano, 1971 (Guaicaipurinae, Nasir et al., 1971; Cathaemasiidae, Fuhrmann, 1928) contains only one species, i.e., G. pseudoconcilia (Nasir, Díaz, and Lemus de Guevara, 1969). The cercariae of this fluke parasitize the freshwater snail, Pomacea glauca (L.), and encyst in the gills of the freshwater fishes, Lebistes reticulatus (Peters) and Rivulus hartii (Boelenger). The metacercariae, 8 days old, were fed to a laboratory-raised pigeon, and adults developed in its cloaca 10 days later at which time eggs appeared in its feces. The necessity for the erection of a new genus, Guaicaipuria, and a new subfamily, Guaicaipurinae, of the family Cathaemasiidae has already been discussed (Nasir, Díaz, and Marcano, 1971). A new species of cercaria, readily distinguished from that of Guaicaipuria pseudoconcilia due to the absence of a digestive tract posterior to the pharynx, was found which failed to encyst in the fishes, Lebistes reticulatus and Rivulus hartii. However, when tadpoles, Engystomops pustulosus (Boettger), were exposed the cercariae readily encysted in the gills. When infected tadpoles were fed to a domestic duckling the corresponding adults developed in its bursa Fabricii. The eggs from

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