

Moisture Stress Effects on Survival of Infective *Trichostrongylus colubriformis* Larvae¹

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Abstract: Water was evaporated from infective *Trichostrongylus colubriformis* larvae suspended in tap, distilled, and triple-distilled water, and the nematodes were then exposed to 50% and 70% relative humidity (RH) at 20 and 30 C. Sample groups were rehydrated for 4 h daily in similar quality water, observed for motility and counted, then returned to the same RH and temp and re-desiccated. Desiccation and rehydration were repeated until all motility ceased. Longest survival was 30 days at 20 C and 70% RH. In all temp and RH combinations, control (nondesiccated) and desiccated larvae survived longer in distilled or triple-distilled water than in tap water. **Key Words:** desiccation, larval survival.

Little attention has been given to the effects of repeated desiccation and rehydration on the survival of trichostrongylid larvae. Andersen and Levine (1) studied the effects of a single desiccation on *Trichostrongylus colubriformis* (Giles, 1892) larvae and made a limited study of the effects of repeated desiccation, and Todd et al. (3) studied the effects of repeated desiccation on infective *Haemonchus contortus* (Rudolphi, 1803) larvae. Laboratory evaluation of the ability of infective *T. colubriformis* larvae to withstand repeated desiccation and hydration was made to correlate with data from studies on the distribution of *T. colubriformis* in various geographical areas.

MATERIALS AND METHODS

The isolate (RLS strain) of *T. colubriformis* used was originally obtained in 1963 from Harry Herlich, USDA Animal Disease Laboratory, Auburn, Alabama and has been maintained by infecting helminth-free sheep at the University of Illinois, College of Veterinary Medicine, Urbana. Feces containing eggs of *T. colubriformis* from monospecifically infected sheep were collected and incubated at 30 C and 100% RH for 6 days. After incubation, third-stage larvae were collected by a modified Baermann technique, cleaned by repeated washing and centrifugation in tap water, then placed in either tap, distilled, or triple-distilled water and diluted to approximately 150 larvae per

ml. One-ml aliquots of this suspension were pipetted into 60 × 15 mm petri dishes and the motile larvae counted. The suspending water was evaporated from the open petri dishes at the following temp and relative humidities (RH): 20 C, 50 and 70% RH; 30 C, 50 and 70% RH. The fluid evaporated in a few hours and the larvae dried out. The petri dishes then were stored under these same conditions. Control samples were subjected to the same temp and RH conditions as the above, except that the petri dishes were kept covered and the larvae were never allowed to dry.

The petri dishes were removed after 1 day, and 1 ml of the same type of water (tap, distilled, or triple-distilled) in which the larvae had originally been desiccated was immediately added to each petri dish. The dishes were kept at approximately 25 C for about 4 h, the motile larvae counted, and returned to the same conditions as before. This procedure was followed until no motile larvae were observed for at least 2 days. In preliminary observations, the highest mortality of larvae occurred during the first 10 days; after that the mortality rate was much lower. Therefore, we counted the motile larvae daily for the first 10 days of repeated daily desiccation and rehydration and then at 2-5 day intervals thereafter.

RESULTS

At all temp the controls and desiccated larvae survived longer in triple and single-distilled water than in tap water (Fig. 1). At 20 C, triple-distilled water samples had significantly longer survival times than distilled water samples (Fig. 1-A, B). At 30 C the survival times were nearly identical for both the triple- and single-distilled water samples (Fig. 1-C, D).

Survival rates appeared to be little affected

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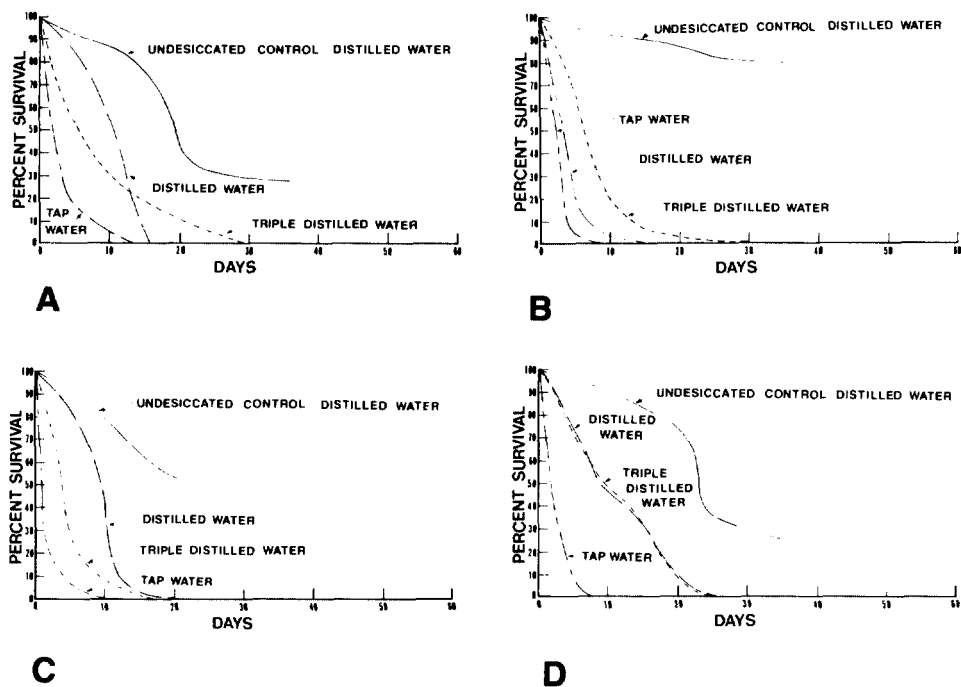


FIG. 1-(A to D). Percent survival of *Trichostrongylus colubriformis* larvae after daily desiccation and rehydration at the indicated temp and relative humidity (RH). A. 20 C, 50% RH; B. 20 C, 70% RH; C. 30 C, 50% RH; D. 30 C, 70% RH.

by RH. At 20 C, 50% and 70% RH single- and triple-distilled samples had nearly identical survival results (Fig. 1-A, B). At 30 C, specimens in triple-distilled water kept at 70% RH survived approximately 10% (3 days) longer than the specimens at 50% RH in triple-distilled desiccants (Fig. 1-C, D).

In triple-distilled water, a higher percentage of larvae survived at 20 C than at 30 C. At 20 C, 70% RH maximum survival was 30 days vs. 25 days at 30 C, 70% RH. At 20 C, 50% RH maximum survival was 29 days vs. 21 days at 30 C, 50% RH. In tap water, maximum survival was greater at 20 C, than at 30 C. At 20 C, 70% RH larvae survived for 9 days while at 30 C, 70% RH they survived 8 days. At 20 C, 50% RH in tap water larvae survived 13 days and at 30 C, 50% RH they survived 10 days. In distilled water the results varied from above. At 20 C and 50% RH larvae survived for 16 days vs. 17 days at 30 C and 50% RH. At 20 C and 70% RH survival was for 14 days vs. 25 days at 30 C and 70% RH.

DISCUSSION

In an earlier study using the same strain of

T. colubriformis, Andersen and Levine (1) found that desiccation of the infective larvae was beneficial to their survival at temp below freezing and at 35-50 C. However, at intermediate temp, larval survival was about as long when desiccated as when kept moist. They used tap water in their studies, whereas we used tap, distilled, and triple-distilled water. Furthermore, we kept the larvae only at 20 and 30 C. Finally, they did not control the RH of storage at the various temp. Their larvae were initially dried at 30 C and 65-75% RH and then placed in storage cabinets at the indicated temp.

After a single desiccation, Andersen and Levine (1) found that *T. colubriformis* infective larvae survived more than 128 days at 20 and 25 C; 28 and 20%, respectively, were alive at 128 days after desiccation. At 35 C, the larvae survived 64 days, at which time 5% were still alive, but none survived 128 days. After repeated daily desiccations and storage at 30 C, they found the larvae survived only 7 days at 65-75% RH and only 4 days at 20-30% RH.

In our study, larvae survived better at 20 C than at 30 C, and at 50% than at 70% RH. In

each case, however, only a small percentage of larvae was alive on the terminal day. We thus confirmed and extended the results obtained from the limited studies of Andersen and Levine (1).

We conclude that repeated desiccation was much more lethal than a single desiccation. An important factor in this difference is probably the increase in salt concentration following repeated desiccation in tap water. However, this factor is probably less important than desiccation itself, since in our study with triple-distilled water, the larvae survived only 20-30 days of repeated desiccation at 20 or 30 C and 50 or 70% RH.

T. colubriformis is a particularly important parasite of sheep in relatively cool summer climates such as those of England and the Pacific Northwest, whereas *H. contortus* replaces it in importance in relatively hot summer climates such as that of Illinois (2). This difference is thought to be due to the differing effects of climate in these locations on the survival of larvae outside the host. A comparison of our findings on the effects of repeated desiccation and rehydration on *T. colubriformis* larvae with those of Todd et al. (3) on *H. contortus* larvae is, therefore, in order. The two studies were carried out under the same conditions and in the same laboratory. In both cases, survival was best when triple-distilled water was used, next best when ordinary distilled water was used, and poorest when tap water was used. This difference was undoubtedly due to the

deleterious effects of increasing amounts of salts resulting from repeated wetting and desiccation.

T. colubriformis infective larvae were not nearly as resistant to repeated desiccation and rehydration as infective *H. contortus* larvae. The longest survival recorded for the former was 30 days at 20 C and 70% RH, whereas the longest survival recorded for the latter was 80 days at 20 C and 75% RH. This difference in response to repeated desiccation may account for the difference in prevalence of the two species in different climates. In Illinois, there is dew practically every night during the summer and the vegetation dries off every day. Conditions are right, therefore, for the larvae to be desiccated and rehydrated every 24 h. The apparent difference between the reaction of infective larvae of *H. contortus* and *T. colubriformis* to repeated desiccation may in part account for the differences in prevalence and distribution of the two parasites.

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