Moisture Stress Effects on Survival of Infective *Haemonchus contortus* Larvae[1]

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Abstract: Water was evaporated from infective *Haemonchus contortus* larvae suspended in tap, distilled and triple-distilled water, and the nematodes were then exposed to 50% and 75% relative humidity (RH) at 20, 30, 40, and 50 C. Sample groups were rehydrated 4 hr daily in similar quality water, observed for motility, then returned to the same RH and temperature and re-evaporated. This was continued until all motility ceased. Longest survival was 80 days at 20 C and 75% RH. In all temperature and RH combinations control (non-desiccated) and desiccated larvae survived longer in distilled or triple-distilled water than in tap water. *Key Words: Haemonchus contortus,* Moisture stress, Survival, Larvae.

Although the survival of infective *Haemonchus contortus* larvae under different environmental conditions has been reported (3, 4, 5, 6, 8, 9), little attention has been given to the effects of repeated desiccation and rehydration. Because some *H. contortus* larvae were found to survive summer conditions at Urbana, Illinois, evaluation of their ability to withstand repeated hydration and desiccation under laboratory conditions was needed to interpret data from field studies.

MATERIALS AND METHODS

The isolate of *H. contortus* used was obtained from Dr. Ray Shumard, Eli Lilly and Company, Indianapolis, Indiana, in 1966 and has been maintained by infecting worm-free sheep at the University of Illinois, College of Veterinary Medicine, Urbana. Feces containing eggs of *H. contortus* from monospecifically infected sheep were collected and incubated at 30 C and 100% relative humidity (RH) for 6 days. After incubation, the 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 200 per ml. One ml aliquots of this suspension were pipetted into 60 × 15-mm petri dishes and the motile larvae were counted under a dissecting microscope. There were three replicates of each experiment. The suspending water was evaporated from the open petri dishes at the following temperatures and RH's: 20 C, 50 and 75% RH; 30 C, 50 and 75% RH; 40 C, 50 and 75% RH; and 50 C, 50 and 75% RH. The fluid evaporated in a few hours so that the larvae dried out. The petri dishes were then stored under these same conditions. Control samples were subjected to the same temperature and RH conditions as the above, except the petri dishes were kept covered and the larvae were never allowed to dry.

The petri dishes were removed after 1 day, and then at daily intervals for 9 more days 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 then kept at approximately 25 C for about 4 hr and the motile larvae were counted; they were then 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. This was the basis for counting the motile larvae daily for

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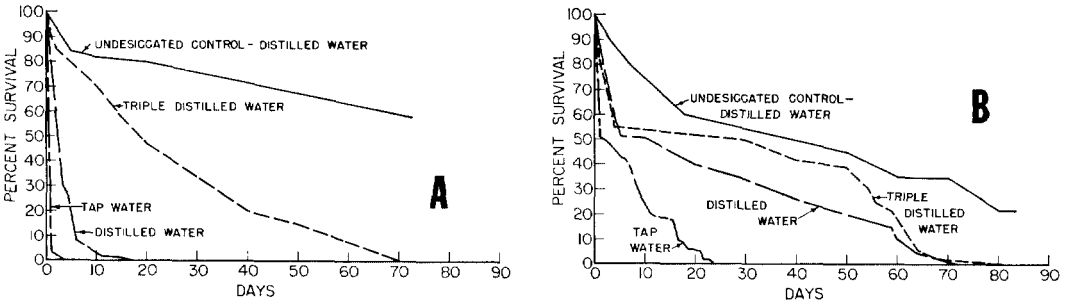

FIG. 1. Percent survival of *Haemonchus contortus* 3rd stage larvae after repeated desiccation and rehydration. **A.** At 20 C and 50% relative humidity; **B.** At 20 C and 75% relative humidity.

the first 10 days of repeated daily desiccation and then at 2–5 day intervals.

RESULTS

At all temperatures the controls and desiccants survived longest in triple-distilled water, followed by distilled and then tap water (Figs. 1 & 2).

The relative humidity to which the larvae were exposed was an important factor affecting survival at 20 C. At 20 C and 50% RH, all larvae desiccated in tap, distilled and triple-distilled water were dead after 3, 17 and 70 days, respectively (Fig. 1-A) while those at 20 C and 75% RH were dead at 24, 72, and 84 days in tap, distilled and triple-distilled water, respectively (Fig. 1-B). Relative humidity had little effect on survival at 30 C. The larvae in tap water were all dead after 8 days at 50% RH (Fig. 2-A) and after 7 days at 75% RH (Fig. 2-B). When larvae were desiccated at 30 C in distilled water at 50% RH, all were dead by 30 days (Fig. 2-A); all were dead by 14 days at 75% RH (Fig. 2-B). In triple-distilled water, all desiccants were dead by 32 days at 50% RH (Fig. 2-A), whereas at 75% RH all were dead by 48 days (Fig. 2-B).

A higher percentage of larvae survived at 20 C than at 30 C, especially at 75% RH (Figs. 1 & 2). When desiccated in tap water at 75% RH, all larvae were dead by 24 days at 20 C and by 7 days at 30 C. In distilled water these figures were 72 days for 20 C and 14 days at 30 C. When desiccated in triple-distilled water, all were dead by 84 days at 20 C and 48 days at 30 C. At 40 C and 50% RH in tap, distilled and triple-distilled water, all larvae were dead by 3, 6 and 6 days, respectively; at 40 C and 75% RH the same values were 4, 7, and 8 days. No larvae survived even 1 day when desiccated at 50 C and 50% or 75% RH.

Because best survival was obtained at 20 C and 75% RH in samples desiccated in distilled water, samples were stored for 1–10 days under these conditions before being wetted and subjected to subsequent daily desiccations and rehydrations. The percentages of survival when larvae were initially desiccated for 1, 3, 5, and 10 days before being first wetted are given in Fig. 3. The percentages of survival for 2, 4, 6, 7, 8, and 9 days of initial desiccation were similar; all larvae were dead by 84, 86, 68, and 84 days when the samples were desiccated initially for 1, 3, 5, and 10 days, respectively. The data obtained from the experiments at other temperatures and humidities were similar, indicating that the length of the entire desiccation period, rather than the number of times desiccation was repeated, was the most important factor in determining survival.

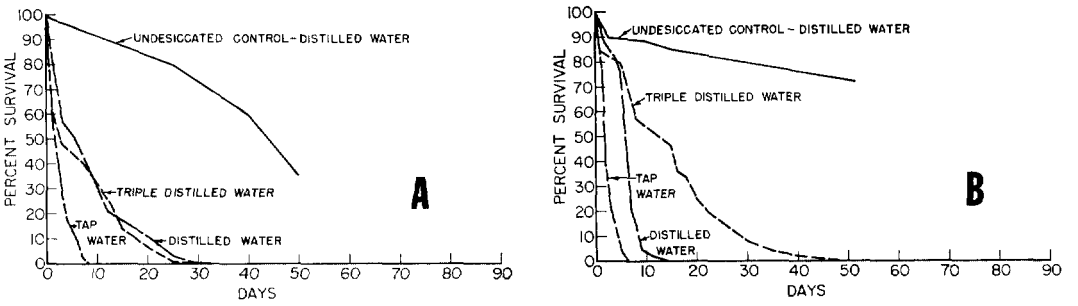

FIG. 2. Percent survival of *Haemonchus contortus* 3rd stage larvae after repeated desiccation and rehydration. **A.** At 30 C and 50% relative humidity; **B.** At 30 C and 75% relative humidity.

DISCUSSION

Because *H. contortus* is an important parasite of sheep in many parts of the world, its ecologic characteristics must be carefully evaluated in order to determine the factors affecting its survival. During the summer at Urbana, Illinois, dew is formed nearly every night. This is often followed by hot days during which the dew evaporates. Field studies on the survival of infective *H. contortus* and *Trichostrongylus colubriformis* larvae have shown that some can survive for long periods (Todd—unpublished data, 2). The ability of the larvae to withstand repeated desiccation is an important factor for their survival.

Poole (7) found that 50% of the infective larvae of *Nematodirus filicollis* survived 10 repeated 5-day desiccations at 22–24 C. At the same temperature, 90% survived 20 daily desiccations. Marquardt, in Andersen and Levine (1), reported that *N. spathiger* larvae survived 39 repeated desiccations and wettings. Andersen and Levine (1) reported that all infective larvae of *T. colubriformis* were dead after 8 consecutive daily desiccations at 30 C and 65–75% RH; at 30 C and 20–30% RH all were dead after 5 daily desiccations. *Haemonchus contortus* larvae are thus considerably more resistant to desiccation than *T. colubriformis* larvae but not as much so as *Nematodirus* larvae.

That *H. contortus* larvae can withstand desiccation under field conditions has been reported by Gordon (3), Mönnig (6) and Rose (8); however, the effects of repeated desiccation have evidently not been reported.

Our data indicate that the initial period of desiccation before the larvae are repeatedly hydrated and desiccated is not an important factor in determining survival. Whether larvae were initially desiccated for 1 or 10 days, all were usually dead within the same 20-day period.

The medium in which the larvae were rehydrated was an important factor. Tap water contains dissolved materials which accumulate in the petri dishes as the daily 1 ml of water was added. This accumulation of salts

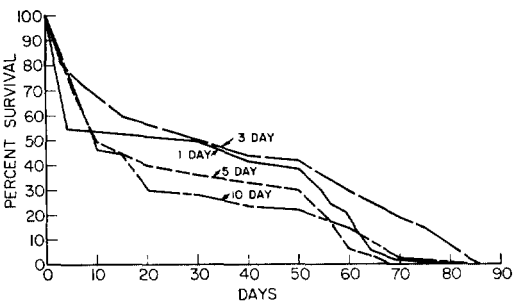

FIG. 3. Percent survival of *Haemonchus contortus* 3rd stage larvae after 1, 3, 5, and 10 days initial desiccation at 20 C followed by repeated daily desiccation at 20 C and 75% relative humidity.

may have been the reason that the larvae died in tap water more rapidly than in distilled and triple-distilled water. Larvae in the non-desiccated control samples also survived longer in triple-distilled water than in tap water or distilled water.

Kates (4, 5) reported that the free-living stages of *Haemonchus* are not as resistant to adverse environmental conditions as are those of some other trichostrongylid genera; however, *H. contortus* is more prevalent than other trichostrongyles in some areas. Factors such as its high reproduction rate and its longevity in the host may account in part for its prevalence. The ability of the infective larvae to withstand repeated desiccation is probably also an important factor for maintaining the species. This factor might account, at least in part, for the better adaptation of *H. contortus* than *T. colubriformis* to midwestern United States conditions.

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