Influence of Cold Temperatures upon Development and Survival of Eggs of Washington Isolates of *Haemonchus contortus* and *Ostertagia circumcincta*

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**ABSTRACT:** Eggs of isolates of *Ostertagia circumcincta* and *Haemonchus contortus* from sheep in eastern Washington were examined for their ability to develop at 10°C, and to survive exposure to −18°C for 15 hr. Eggs of *O. circumcincta* were first larvated after 48 hr of incubation at 10°C, and 95% of the eggs were larvated after 120 hr of incubation. Eggs of *H. contortus* developed less rapidly with only 32% larvated after 120 hr of incubation. Survival of eggs (determined by their ability to hatch) after exposure to −18°C was >87% for *O. circumcincta* and <4% for *H. contortus*. These results indicate that the eggs of the strains of *O. circumcincta* and *H. contortus* examined differed in their ability to tolerate cold temperatures and that those of the former should be more successful than those of the latter in overwintering in colder climates.

The capacity of free-living stages of trichostrongylid nematode parasites to survive exposure to cold temperatures has been examined by many investigators (Furman, 1944; Kates, 1950; Crofton, 1965; Gibson and Everett, 1972, 1976; Todd et al., 1976; Le Jambre, 1981); however, strains of parasites from the northwestern United States have not been included in these observations.

The present study examines differences in survival of eggs and larval maturation of eastern Washington isolates of *Haemonchus contortus* (Rudolphi, 1803) and *Ostertagia circumcincta* (Stadelmann, 1894) subjected to cold temperatures. Observations included the ability of eggs to develop at 10°C and to hatch after exposure to −18°C. The amount of lipid present in eggs also was measured to determine whether this energy source (Passey and Fairbairn, 1957; Ward and Fairbairn, 1970; Kahn and McFadden, 1980; Womersley et al., 1982) could be correlated with cold survival.

**Materials and Methods**

Nematode eggs were obtained from donor lambs infected with strains of either *H. contortus* or *O. circumcincta* isolated from naturally infected sheep from the Palouse region of eastern Washington. The lambs were raised in confinement until 10 weeks of age. Prior to experimental infection, it was demonstrated by fecal examination that the lambs were free of nematode infection. Lambs were infected by oral administration of approximately 2,000 L₂ of *H. contortus* or 10,000 L₂ of *O. circumcincta*. Donor lambs were maintained in raised stanchions and given free access to pelleted alfalfa hay (WSU sheep ration 7008) and water. Specificity of infection was monitored by examination of larvae from fecal cultures obtained when infections became patent and at 1- to 2-week intervals thereafter.

The development of eggs from fecal pellets incubated at 10°C was evaluated by determining when nuclear indentations and larval stages first appeared and subsequently increased in numbers. The percentage of such eggs in these cultures was recorded at 24- to 48-hr intervals for 168 hr (Table 1). A sugar flotation technique (Cox and Todd, 1962) was used to separate eggs from feces for microscopic examination, which was used to assign them into 3 categories based on the appearance of their nuclei: (1) oval nucleus, with 8–500 cells; (2) indented nucleus, with a visible ventral indentation; (3) larvated, with tadpole or prehatch larva (Christie and Jackson, 1982). Two to 4 replicates were performed for each interval reported.

The ability of eggs to survive intense cold was evaluated by determining the percentage of eggs that hatched after exposure to −18°C for 15 hr. In the procedure used, feces containing eggs were incubated at −18°C, and eggs were removed from the feces by sugar flotation and then incubated in distilled water at 30°C for 24 hr to stimulate hatching. The percentage of hatching then was determined immediately by viewing the samples with a dissecting microscope and counting intact eggs and hatched larvae. At least 3 aliquots each containing >100 eggs and larvae were counted for all samples. Two replicates were performed for both *H. contortus* and *O. circumcincta* in all treatment and control groups (Table 2).

Both fresh and developing eggs were tested. Developing eggs were incubated at 10°C in feces for 48 hr (*O. circumcincta*) to 96 hr (*H. contortus*) prior to exposure to −18°C. The incubation periods were based on the approximate times when larvated eggs first ap-

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peared in 10°C cultures (Table 1). Controls included both fresh and developing eggs that were not exposed to −18°C.

The lipid content of fresh eggs was measured in 4 experiments. Eggs of *H. contortus* and *O. circumcincta* were cleaned of fecal debris by sugar flotation and washing in distilled water and were used immediately in paired experiments under identical conditions. Lipid content of each sample tested was determined as follows: (1) a known number (>1,000) of eggs was placed in a test tube and treated with 0.5% hypochlorite for 10 min, rinsed twice in distilled water, centrifuged at 5,000 rpm for 1 min, rinsed twice in 70% ethanol, and transferred to glass slender dishes; (2) eggs were stained for 2–3 hr in 70% ethanol saturated with Sudan IV; (3) excess stain was removed with a pipet, the eggs were rinsed twice in distilled water, and the absorbed stain was extracted by disrupting the eggs in 100% ethanol in a Heat Systems-Ultrasonics sonicator at 50 W for 3 min; (4) micrograms of lipid per milliliter of ethanol were estimated by measuring absorbance of the supernatant at 515 nm with a Varian DMS 80 spectrophotometer; (5) absorbance was compared with a standard absorbance curve produced with corn oil saturated with Sudan IV, and total lipid per sample was converted to µg of lipid per 1,000 eggs. Egg size of each species was determined by measuring length and width of 25 eggs obtained from fresh fecal pellets using a compound microscope equipped with an ocular micrometer at 400×.

The Student's *t*-test (Steel and Torrie, 1980) was used to test significance of differences. Data from all experiments were treated as unpaired observations, except those involving lipid measurements, which were treated as paired observations.

**Results**

Eggs of both *H. contortus* and *O. circumcincta* developed at 10°C (Table 1); however, larval stages of *O. circumcincta* were present earlier and in much higher proportions than those of *H. contortus* in samples taken after 24 hr of incubation. Fresh and developing eggs of *O. circumcincta* also were superior in their ability to withstand exposure to −18°C for 15 hr (Table 2). Mean percentage of hatching for *O. circumcincta* was from 88 to 93%, whereas that of *H. contortus* was from 1 to 4%. After 24 hr of incubation at 30°C, most (86–95%) fresh eggs of both parasites hatched, but significantly (*P < 0.01*) fewer developing eggs of *H. contortus* hatched than did those of *O. circumcincta*.

Other results indicated that: (1) there was significantly (*P < 0.05*) more lipid in eggs of *O. circumcincta* (126 µg/1,000 eggs) than in eggs of *H. contortus* (75 µg/1,000 eggs); and (2) eggs of *O. circumcincta* were significantly (*P < 0.05*) larger than those of *H. contortus*, measuring 87.5 ± 3.4 × 47.7 ± 2.2 µm and 73.2 ± 5.0 × 43.0 ± 1.7 µm, respectively.

**Table 1. Development of eggs of *Haemonchus contortus* and *Ostertagia circumcincta* at 10°C.**

<table>
<thead>
<tr>
<th>Incubation (hr)</th>
<th>Replicates (no.)</th>
<th>Eggs examined</th>
<th>oval nucleus</th>
<th>indented nucleus</th>
<th>larvated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemonchus contortus</em></td>
<td>0</td>
<td>4</td>
<td>4,727</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>3,958</td>
<td>&gt;99</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>5</td>
<td>2,580</td>
<td>78</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>4</td>
<td>2,121</td>
<td>44</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>2</td>
<td>765</td>
<td>9</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>2</td>
<td>752</td>
<td>11</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>2</td>
<td>1,659</td>
<td>16</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td><em>Ostertagia circumcincta</em></td>
<td>0</td>
<td>4</td>
<td>1,803</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>1,528</td>
<td>98</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>5</td>
<td>1,538</td>
<td>59</td>
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<tr>
<td>72</td>
<td>4</td>
<td>1,413</td>
<td>15</td>
<td>56</td>
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<tr>
<td>96</td>
<td>2</td>
<td>609</td>
<td>2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>2</td>
<td>513</td>
<td>2</td>
<td>3</td>
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<tr>
<td>168</td>
<td>2</td>
<td>611</td>
<td>1</td>
<td>4</td>
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</tbody>
</table>

Routine monitoring of larvae from fecal cultures from donor lambs indicated that infections of *H. contortus* and *O. circumcincta* were essentially monospecific. Over 99% of larvae in all cultures examined were those of the intended species.

**Discussion**

The results of the present experiments demonstrate differences in response to cold temperatures in fresh and developing eggs of strains of *O. circumcincta* and *H. contortus* isolated from eastern Washington. Most (>87%) eggs of the former developed rapidly at 10°C and hatched readily after exposure to −18°C. Eggs of the latter developed slowly and incompletely at 10°C and few (<4%) fresh or developing eggs hatched after exposure to −18°C. Although the mechanism responsible for cold tolerance was not determined, it seems unlikely that the egg shell could be an effective insulator at the temperatures tested. It is more probable that the eggs of *O. circumcincta* and *H. contortus* contain differing amounts of a cryoprotectant, such as trehalose, which serves this purpose in free-living stages of *Nematodirus battus* as reported by Ash and Atkinson (1982).

Sustained viability of eggs subjected to cold also may depend upon their content of energy stores. The fact that the larger eggs of *O. circumcincta* contained nearly 40% more lipid than did the smaller eggs of *H. contortus* suggests that this
energy source enhances cold survival. This hypothesis is supported further by observations of Ash and Atkinson (1982), who have shown that the large eggs of Nematodirus spp. are extremely cold-resistant.

The practical importance of the results is that they advance understanding of the role eggs of H. contortus and O. circumcincta play in the epidemiology of these parasites in the Northwest. Development at 10°C of the strain of H. contortus used in the present study was more like that observed by Le Jambre (1981) for a strain (H. contortus cayugensis) of this parasite from New York than for strains of Haemonchus from warmer areas. However, the degree of cold resistance demonstrated was not sufficient to allow the egg stages of this parasite to make a major contribution to overwintering on pastures in eastern Washington, where temperatures frequently fall below −18°C. On the other hand, the egg stages of the strain of O. circumcincta tested were extremely resistant to cold temperatures and appear to be capable of surviving local winters.

Acknowledgments
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Literature Cited
Furman, D. P. 1944. Effects of environment upon the free-living stages of Ostertagia circumcincta (Stadelmann) trichostrongylidae: I. Laboratory experiments. American Journal of Veterinary Research 5:79-86.
Todd, K. S. Jr., N. D. Levine, and P. A. Boatman. 1976. Effect of temperature on survival of free-


New Index of Literature on Nematoda Parasitic in Animals

The last of the publications of the Index-Catalogue of Medical and Veterinary Zoology, "Special Publication No. 6: Nematoda and Nematode Diseases," is now available free of charge to those willing to pay the shipping costs. Higher taxa are listed alphabetically to genus level, but specific and subspecific names are listed only under the generic alphabetization. References to the literature include only author and date. The Author Catalogues of the Index-Catalogue of Medical and Veterinary Zoology must be consulted to obtain complete references. The publication period covered in Special Publication No. 6 is 1920–1964. Nematode literature published prior to 1920 was indexed in the Roundworm Catalogue compiled by C. W. Stiles and A. Hassall (published as Hygienic Laboratory Bulletin No. 114). This 1920 Roundworm Catalogue has been reprinted and is also available for the cost of shipping charges. References published after 1964 are available in the Nematoda sections of the Index-Catalogue (Supplements 15–24). Supplements 15–23 are available as above. Supplement 24 is available only from Oryx Press, 2214 North Central at Encanto, Phoenix, Arizona 85004 (Telephone 602-254-6156).

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