# Changes in Morphology of
## Trichostrongylus colubriformis Eggs and Juveniles Caused by Bacillus thuringiensis israelensis[1]

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*Abstract:* Eggs and rhabditiform juveniles of the ruminant parasite *Trichostrongylus colubriformis* developed normally in *Caenorhabditis briggsae* Maintenance Medium. A toxin from a crystal-enriched preparation of the bacterium *Bacillus thuringiensis israelensis* was lethal to nematode eggs and juveniles within 24 hours and to eggs and juveniles after 24 hours of development. Treated eggs had refractive granules and development was arrested, whereas nontreated eggs developed normally. Eggs treated after 24 hours of development contained juveniles that were granulated, had esophageal derangements, and were moribund or dead. The ovicidal toxin from *B. t. israelensis* may facilitate microbial control of parasitic nematodes.

*Key words:* *Trichostrongylus colubriformis*, egg lethality, *Bacillus thuringiensis israelensis*, microbial toxin.

Several *Bacillus* species are insect pathogens and produce insecticidal toxins, but *B. thuringiensis* is the most widely used commercially available bacterium for insect control (6). One subspecies, *B. t. israelensis*, is effective against dipteran insects, such as mosquitos and blackfly larvae, which transmit human parasites. The insecticidal toxin of *B. t. israelensis*, the delta-endotoxin, is a protein which apparently affects membrane lipids (7,8) in the insect gut epithelium and changes membrane permeability (5).

An unidentified toxin from *B. t. israelensis* is lethal in vitro to eggs of *Trichostrongylus colubriformis* and six other species of nematodes (3). An alteration of permeability of the egg may be responsible for the toxicity (1).

This study presents the effects of *B. t. israelensis* on the morphology of *T. colubriformis* eggs and newly hatched juveniles. Closer examination of structural changes in eggs and juveniles may contribute to our understanding of the toxin's mode of action.

## MATERIALS AND METHODS

A crystal-rich fraction of *Bacillus thuringiensis israelensis* was prepared by manual grinding and sonication in reagent-grade water as described previously (3). Axenic eggs of *T. colubriformis* were obtained by density gradient centrifugation and surface sterilization with sodium hypochlorite as described previously (1). Several thousand eggs in reagent-grade water were placed in sterile 50-ml screw-capped Erlenmeyer flasks containing *Caenorhabditis briggsae* Maintenance Medium (CbMM, Gibco). *B. t. israelensis* (2.7 µg total protein/ml) was added to give a final volume of 2 ml. Flasks were incubated at 22 C for 24 hours to allow development and hatching of juveniles. Other flasks with eggs were incubated for 24 hours before adding *B. t. israelensis* and incubating for an additional 24 hours. Control flasks with eggs and juveniles were handled similarly, but received only reagent-grade water rather than toxin. Control and treated eggs and juveniles were examined microscopically and photographed with a Zeiss M63 photomicrographic system. Triplicate flasks were used for each control or treatment, and several hundred eggs were observed from each flask.

## RESULTS

After 24 hours, *T. colubriformis* eggs from nontreated flasks developed normally (Fig.

1). Fully formed rhabditiform juveniles were moving actively within the eggshell. Eggs from flasks incubated with *B. t. israelensis* for 24 hours were not viable (Fig. 2). The embryos did not develop and were condensed with numerous refractive granules visible within the degenerated embryo. Eggs from flasks incubated for 24 hours before treatment with *B. t. israelensis* contained developed, inactive, dead juveniles (Fig. 3). The granular appearance of these eggs obscured the internal morphology of the juveniles, such as the intestinal lumen seen in Figure 1.

Newly hatched, nontreated juveniles of *T. colubriformis* exhibited normal morphology after incubation for 24 hours (Fig. 4). In contrast, after 24 hours of incubation juveniles from cultures treated with *B. t. israelensis* were moribund or dead (Fig. 5). These juveniles had refractile granules throughout their entire length, and their intestinal cells apparently had degenerated. Most internal morphology, with the possible exception of the anterior esophageal lumen, was obscured by these granules. Juveniles from nontreated flasks that were incubated for 48 hours (Fig. 6) were active and had an internal morphology similar to juveniles from 24-hour control flasks (Fig. 4). However, the digestive tract was narrower from the isthmus of the esophagus to the anus in the 48-hour control juveniles than in 24-hour control juveniles. This decrease in width created a visible gap between the intestine and the body wall. Juveniles from flasks that were treated with *B. t. israelensis* after 24 hours of incubation were moribund or dead when examined after 48 hours of incubation (Fig. 7). The gap between the body wall and intestinal tract was more pronounced in the esophageal region of treated juveniles. The lumen of the intestine was obscured by refractile granules. These granules were visible also throughout the length of the juveniles, although not to the extent observed in juveniles initially treated with *B. t. israelensis* and observed after incubation for 24 hours (Fig. 5). Many juveniles treated with *B. t. israelensis* on the first or second
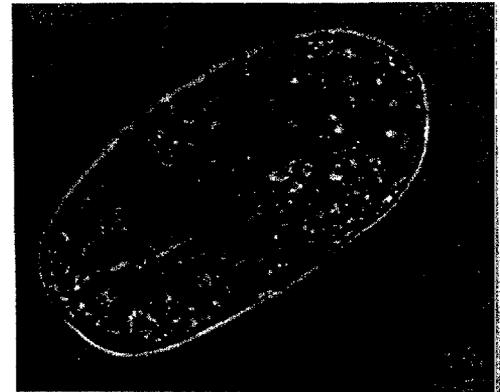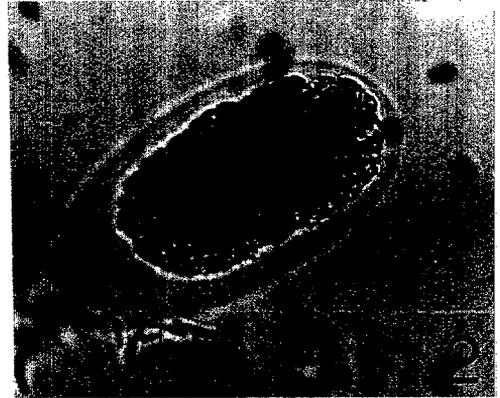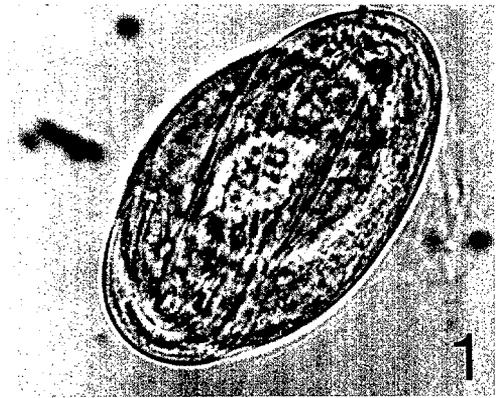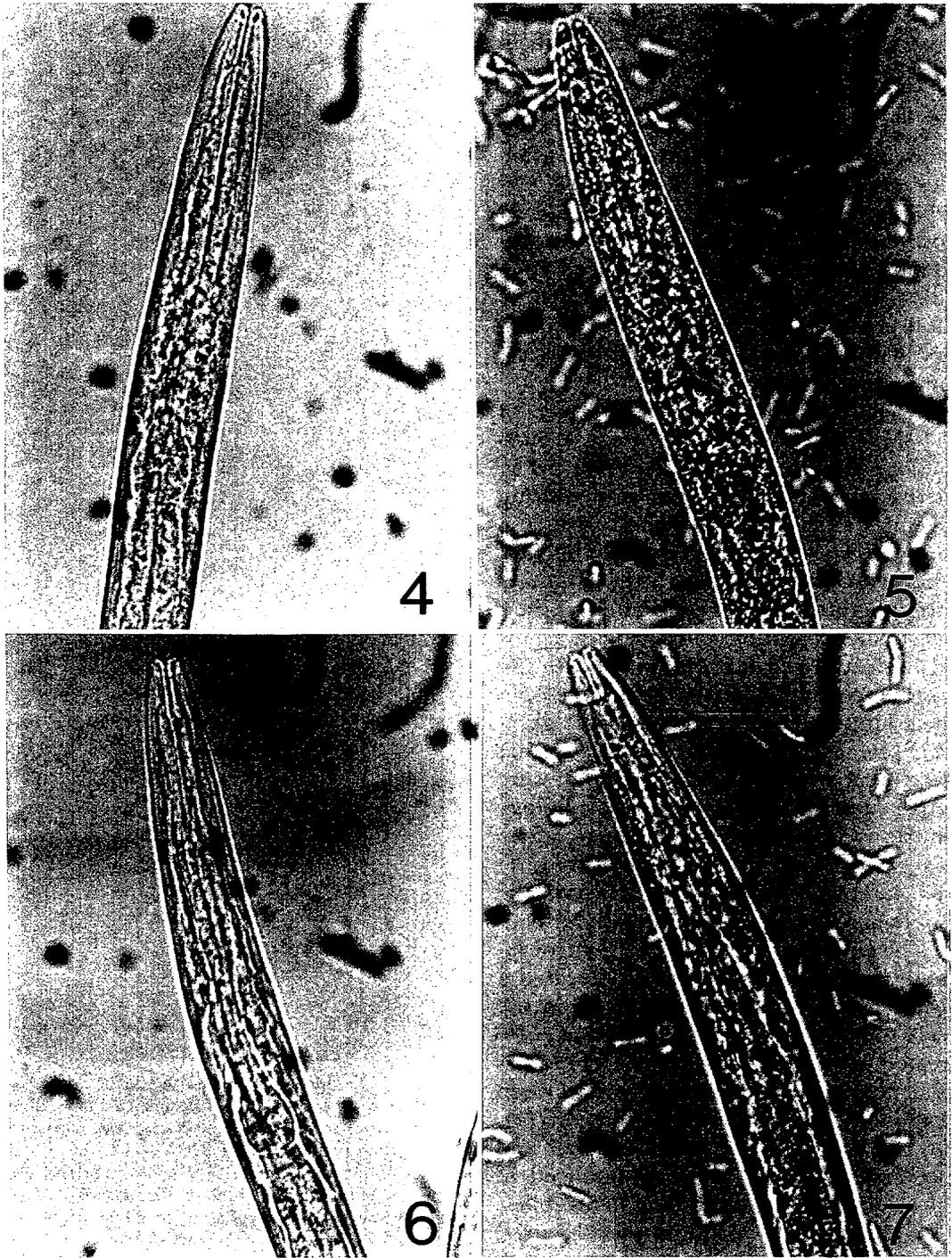


FIGS. 1–3. Eggs of *Trichostrongylus colubriformis* (×1,062) with and without exposure to a crystal-enriched preparation of *Bacillus thuringiensis israelensis* (2.7 μg total protein/ml) at 22 C. 1) Untreated egg after 24-hour incubation. 2) Treated egg showing granulation after 24-hour incubation with *B. t. israelensis*. 3) Treated egg incubated for 24 hours before addition of *B. t. israelensis* for an additional 24-hour incubation.

day of development had a distorted esophagus (Fig. 8).

## DISCUSSION

Disruption of cell membranes or layers of the eggshell of *T. colubriformis* and sub-

FIGS. 4–7. Rhabditiform juvenile of *Trichostrongylus colubriformis* (×1,062) with and without exposure to a crystal-enriched preparation of *Bacillus thuringiensis israelensis* (2.7 µg total protein/ml) at 22 C. 4) Untreated juvenile after 24-hour incubation. 5) Treated juvenile showing granulation after 24-hour incubation with *B. t. israelensis.* 6) Untreated juvenile after 48-hour incubation. 7) Treated juvenile incubated for 24 hours before addition of *B. t. israelensis* for an additional 24-hour incubation.

FIG. 8. Rhabditiform juvenile of *Trichostrongylus colubriformis* showing distorted esophagus afer incubation for 24 hours with a crystal-enriched preparation of *Bacillus thuringiensis israelensis* (2.7 µg total protein/ml).

sequent cytolysis would release lipid granules for dispersion in the egg or juveniles as seen in Figures 2, 3, 5, and 7. The visible gap between the intestine and body wall of juveniles incubated for 48 hours (Figs. 6, 7) may result from the reduction in intestinal size as the juveniles utilized their available lipid reserves over the incubation period. The dispersion of lipid granules throughout the juveniles in Figures 5 and 7 suggests that the *B. t. israelensis* toxin has contacted the intestinal cells, most likely via ingestion, resulting in cellular degeneration and lipid release.

The abnormalities observed in *T. colubriformis* eggs (Figs. 2, 3) indicate the eggshell was permeable to the toxin which then interacted with the membranes of the em-

bryo (Fig. 2) or caused membrane degeneration after ingestion or permeation through the cuticle of the first-stage juvenile (Fig. 3).

Nutritional deficiencies, such as deletion of cholesterol from the culture media or azasteroid inhibition of cholesterol metabolism, caused morphological alterations in juveniles of *Nippostrongylus brasiliensis* and *Nematospiroides dubius* (2,4). The structural changes in juveniles of *T. colubriformis* after treatment with *B. t. israelensis* were similar to those caused by cholesterol deficiency. Degenerated intestinal cells and lipid granule dispersion seen in *T. colubriformis* juveniles were noted also in *N. brasiliensis* and *N. dubius* and were attributed to disruption of normal membrane integrity.

Interaction of *B. t. israelensis* toxin with cholesterol cannot be directly associated with cell disruption. The morphological abnormalities reported here, however, may suggest that the action of *B. t. israelensis* toxin on nematode eggs is similar to that of *B. t. israelensis* delta-endotoxin on insect cells. Thomas and Ellar (8) proposed that the delta-endotoxin interacted with plasma membrane lipids of dipteran cells and caused a detergent-like rearrangement of the lipids, disruption of the membrane, and cytolysis. The toxic effects of *B. t. israelensis* on nematode eggs and juveniles may result in development of a microbial-based scheme for helminth control.

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