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Clinical History: Multiple instar waxworm (Galleria mellonella) larvae were inoculated with Shigella sp. via injection through a posterior proleg directly into the hemocoel.

Necropsy Findings: Multifocal to diffuse areas of brown discoloration are present on the cuticles of affected and dead larvae.

Gross and Microscopic Images:

Figure 1. Instar pupae cuticles. Gross photo (photo borrowed from www.waxworm.com). Multifocal to coalescing cuticular melanization as represented by the dark areas.
Figure 2. Effacing the fat body (A) are inflammatory nodules (N) containing basophilic bacterial colonies. 200X, H&E stain.

Figure 3. A thrombus (T) is expanding the dorsal vessel and pericardial cells (PC), which are lining the outer tunic, appear to be migrating in response to the intraluminal thrombus. 100X, H&E stain.
Figure 4. Enterocytes (E) of the midgut are necrotic and lined by a biofilm (B) that is adjacent to an area of coagulated (C) eosinophilic hemolymph and areas of melanization. 200X, H&E stain.

Figure 5. The subcuticular region is eroded by a biofilm of bacteria, with adjacent coagulation and melanization (CM) of the fat body. 200X, H&E stain.
Gross and Microscopic Morphologic Diagnoses (based on the pictures shown):

1. Multifocal to diffuse cuticular melanization (Figure 1, Gross photo).
2. Fat body: Inflammatory nodule, focal, marked, with intranodular bacilli (Figure 2, Microscopic photo).
3. Dorsal vessel: Thrombus, focal, marked, with pericardial cell migration (Figure 3, Microscopic photo).
4. Midgut: Enteritis, necrotizing, focally extensive, marked, with bacterial biofilm, coagulation, and melanization (Figure 4, Microscopic photo).
5. Cuticle: Erosion, focally extensive, marked, with bacterial biofilm, coagulation, and melanization (Figure 5, Microscopic photo).

Comments: The purpose of this diagnostic exercise is to introduce the waxworm as an inexpensive alternative to animal models when studying bacterial virulence factors involving the innate immune system (waxworms lack adaptive immunity). Waxworms can be studied in large numbers, they require little space, they have short reproductive cycles, they can be studied at human body temperature (37°C), and they do not require IACUC approval. There are a number of published studies involving numerous bacterial pathogens including, but not limited to, *Shigella* spp., *Streptococcus suis*, *Campylobacter jejuni*, *Actinobacillus pleuropneumoniae*, *Clostridium difficile*, and yeast.

Cell-mediated response: The cell-mediated immune system of the waxworm consists of circulating hemocytes comprising plasmatocytes, granulocytes, spherulocytes, oenocytes, and adipohemocytes. The primary function of the hemocytes are phagocytosis, nodule formation, and encapsulation during the immune response.

In healthy larvae, hemocytes concentrate in the subcuticular area, in the hemolymph, and in close association with the aero-digestive tract, which the investigators describe as functioning like human bronchus-associated lymphoid tissue (BALT) and mucosa-associated lymphoid tissue (MALT). Upon infection, BALT and MALT hemocytes increase where the pathogen is concentrated whereas hemocytes in circulation are reduced in numbers.

Humoral response: The humoral response is orchestrated by soluble effector molecules that immobilize or kill the pathogen and includes complement-like proteins, melanin, and antimicrobial peptides. Melanization is an important part of the humoral response and the most visible indicator that the waxworm is succumbing to the infection. The melanization response can be described as the synthesis and deposition of melanin to encapsulate pathogens at the wound site followed by hemolymph coagulation and opsonization and is analogous to abscess formation in mammalian infections. Last but not least, waxworms also have the ability to opsonize pathogens, secrete antimicrobial agents, and form extracellular nuclear traps in their defense against pathogens.
References:


*The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation. These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the CL Davis website ([http://www.cldavis.org/diagnostic_exercises.html](http://www.cldavis.org/diagnostic_exercises.html)).

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