RESEARCH NOTE

Angiostrongylus costaricensis Life Cycle: a New Proposal

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Abdominal angiostrongiliasis is a disease caused by *Angiostrongylus costaricensis* (Nematoda: Angiostrongylidae) (P Morera & R Cespedes 1971 *Rev Biol Trop 18:* 173-185) and was first reported in humans. The worms live in ileo-colic branches of mesenteric arteries of the vertebrate hosts. The main hosts, *Sigmodon hispidus* and *Rattus rattus* (P Morera 1971 *Bol Chileno Parasitol 25:* 133-134) and Verocinellidae slugs (P Morera & LR Ash 1971 *Bol Chileno Parasitol 25:* 135, C Graeff-Teixeira et al. 1989 *Mem Inst Oswaldo* Cruz *84:* 65-68) have been found in several Latin American countries.

The life history of *A. costaricensis* was described in rats. According to P Morera (1973 *Am J Trop Med Hyg 22:* 613-621) the L3 after their penetration into the intestinal wall, develop in abdominal lymphatic system to L4 and L5. The young adult worms then migrate from the lymphatic vessels to the mesenteric arteries, their definitive habitat, penetrating through the artery walls. However, P Morera statement on the life cycle of *A. costaricensis* did not explain the following critical points: (1) Why is the *A. costaricensis* cycle so different from other metastrongylides worms, which pass through the pulmonary circulation after leaving the lymphatic system? (2) How to explain the P Morera hypothesis of the passage from lymphatic to arterial system through a penetration in mesenteric muscular arteries from the adventitia side, which is a phenomenon not observed in any kind of parasitic disease? (3) How to explain the presence of larvae in the lungs and liver, specially in the portal veins?

In order to elucidate these questions on migratory pathways of *A. costaricensis*, Swiss mice were orally infected with L3 obtained from *Sarasinula* sp. slugs by modified GD Wallace and L Rosen method (1969 *Malacologia 7:* 427-438).

Animals that received 500 L3 were sacrificed 6, 12 hr, 1, 2, 3, 4, 5 and 6 days after infection. Mice infected with 8 L3 were studied on 7, 10, 14, 18, 21, 24, 28, 31, 35 and 40 days after infection (6 mice/point of infection). Specimens from brain, thymus, heart, lungs, liver, stomach, spleen, intestine, mesentery, kidneys, supra-renal and genital apparatus were fixed in formalin-Millonig and embedded in paraffin. Sections were stained in H&E, Weigert's resorcin-fuchsin and Lennert's Giemsa. Intestine and mesentery were also studied by serial sections. To study the L3 penetration, fourteen animals were anaesthetized with cetamine (10mg/kg) supplemented with ether. Abdominal cavity was exposed and a 4 cm long segment of small intestine was closed in its ends without cutting it off from the organ. After injection of 1000 L3 at one of the extremities, the intestine was put back in the abdominal cavity and covered with gaze absorbed in PBS. The intestinal segments were removed at 1, 2, 3, 4, 5, 7, 10, 15, 20, 25, 30, 35, 40 and 45 min after injection and their intestinal contents were washed with PBS to observe the residual larvae. The intestinal walls were fixed in formalin-Millonig, embedded in JB4. Semithins were cut in supercut (2065 Leica Instruments) and stained in H&E.

We obtained the following results: the first step of L3 penetration in the intestinal mucosa was expressed by adhesion on and depression of epithelial cells (4 min). At 5 min, the larvae reached the central lymphatic vessels of the villosities and from 7 min on, some of them were found inside venous vessels of submucosa. From lymphatic vessels the L3 took away to mesenteric lymph nodes where suffered the 3rd molt. L4 left the lymph nodes and by lymphatic thoracic duct reached the venous vessels. After passing through the pulmonary circulation, where they were detected from 3rd to 11th day, they returned to heart, being carried to arteries of various organs by systemic circulation (brain, kidney, ovarius, spleen, stomach). Afterward the worms developed maturity mainly in the mesenteric, pancreatic and ileo-colic arteries (9th day after infection). Although ovules surrounded by spermatozoids were observed in female worms uterus on 9th day, embrionated eggs trapped in small branches of mesenteric artery were found for the first time only since 14th day on. L1 were seen from the 22th day after infection. Egg embrionation occurred preferentially in the me-

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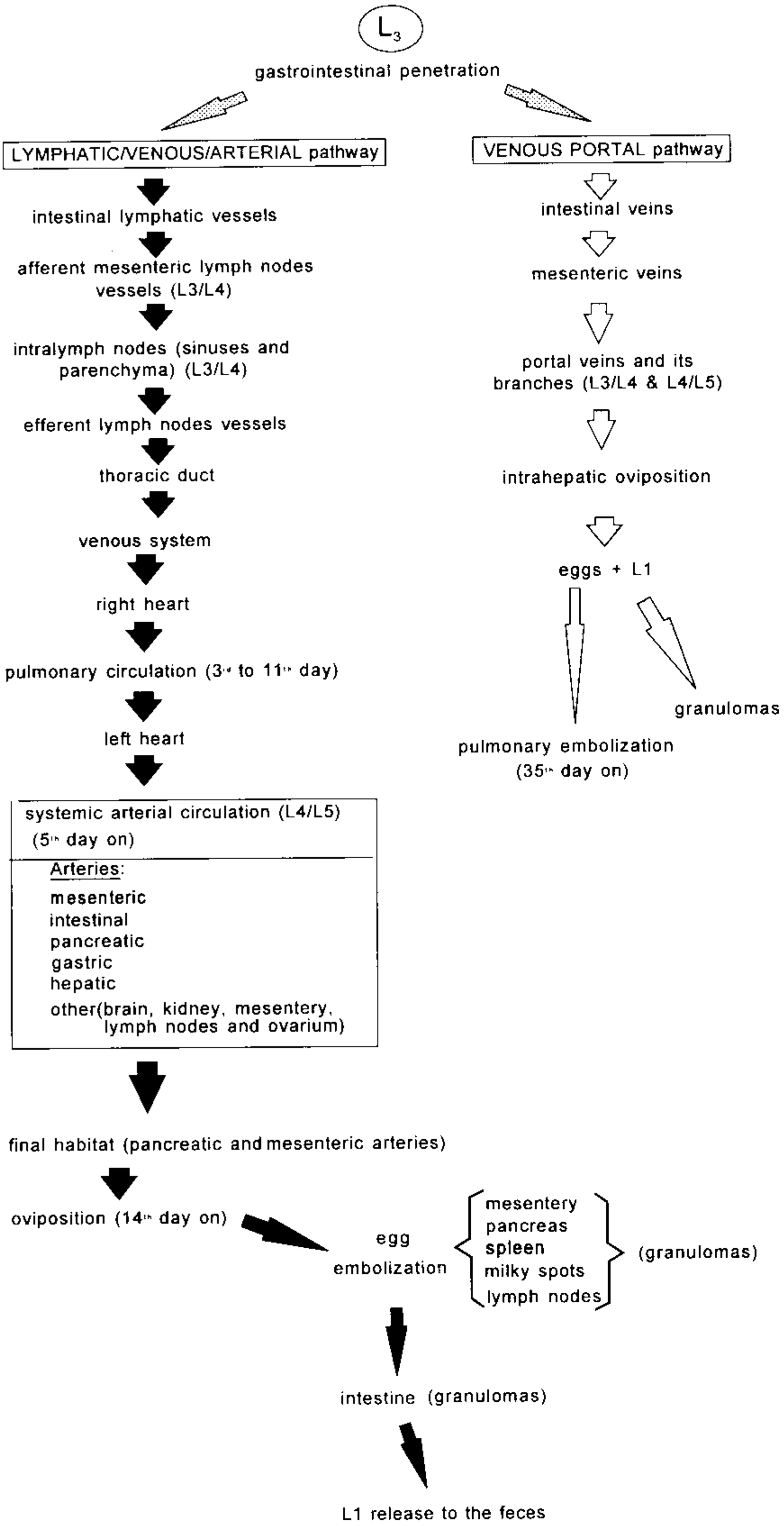

Angiostrongylus costaricencis migration pathway in definitive hosts (Swiss mice).

sentery, lymph nodes, pancreas, liver and milky-spots.

Otherwise, larvae which entered in veins of intestinal wall, reached the intrahepatic portal veins, where they suffer the 3rd and 4th molts, independently from the larvae that migrated through lymphatic-veins-pulmonary and systemic circulation (Fig.). The adult worms that developed in the hepatic venous habitat, lay down eggs that disseminate to portal venous branches and can embolize to the lungs from the liver.

This work shows that *A. costaricensis* migrates by a circulatory pathway to its definitive habitat like other metastrongylides, using the pulmonary circulation to pass from the lymphatic/venous to arterial system. We did not observe larvae entering the mesenteric arterial vessels, direct from the lymphatic. Liver also appears to be an important site of the worm development and maturity. Based on these findings we propose a new life cycle of *A. costaricensis* in the definitive host, different from the classical model, which consists of two possible vascular pathways during the development and maturation of parasites in the mouse model: LYMPHATIC/VENOUS-ARTERIAL and VENOUS PORTAL PATHWAYS. As the L3 developed to adult worms inside the branches of portal veins, the migration to the liver is not considered by us as a "larva migrans" event.