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Localized immunity to the large roundworm *Ascaris suum* in swine

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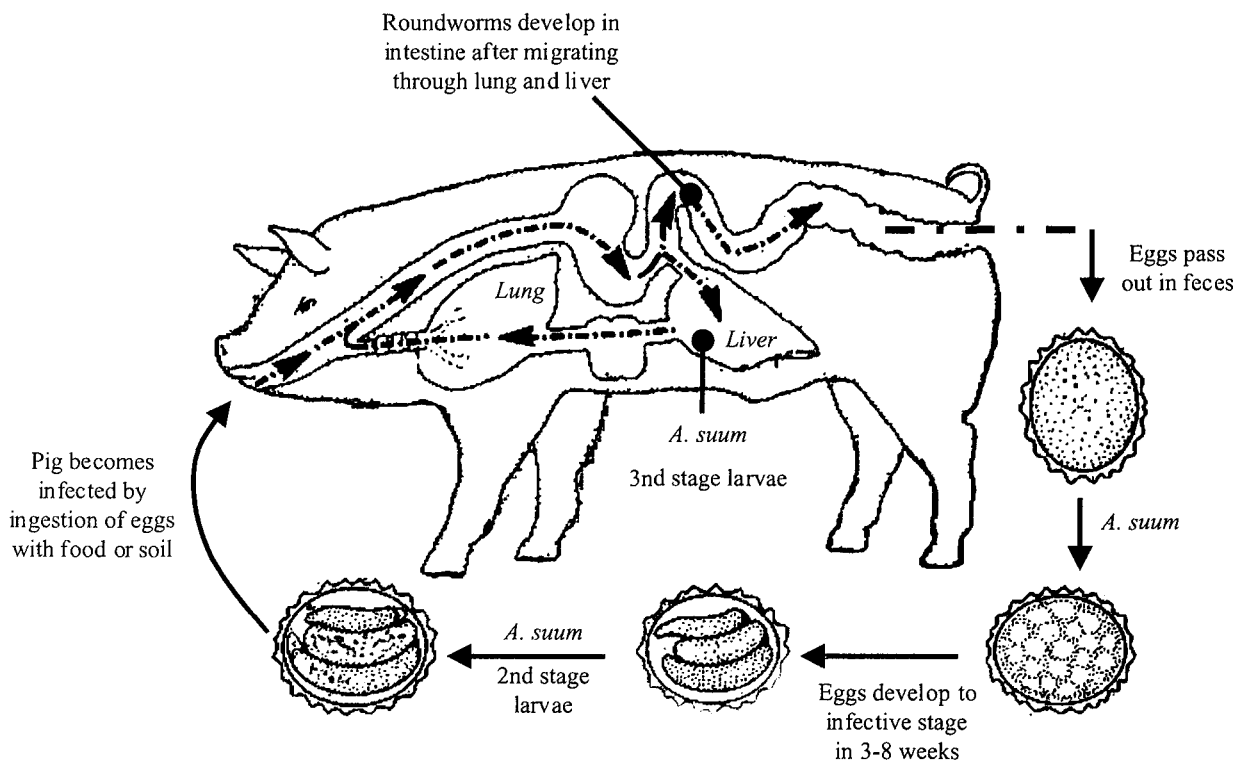
Ascaris suum is the most ubiquitous nematode infection of swine worldwide. These large roundworms are usually found in growing pigs; fewer are found in older pigs indicating the development of acquired immunity following infection. Prevalence varies greatly and is dependent on the type of swine housing, management, and nutrition. Infection with *A. suum* is commonly associated with pastures and outdoor production systems, but it is also a problem in total confinement facilities that do not apply control strategies to reduce parasite exposure (Corwin 1993). The reason for continual infection in these units is the persistence of environmentally resistant infective eggs and high adult worm fecundity with greater than 10^5 eggs shed per female worm per day. Even low level *A. suum* infections can depress feed intake and daily gain producing an increase in feed-to-gain ratio (Murrell 1986, Hale 1985). But what may be of greater importance is that there is skewing of the immune response that protects against extracellular parasites at the expense of reduced effectiveness against intracellular pathogens (Urban et al. 1998). There are many estimates of economic impact of this parasitic infection on the pork-producing industry, but general agreement is that there is significant negative cost to swine production (Stewart and Hale 1988; Kennedy et.al.1988; Steward 1996).

Ascaris suum has a complex single host life cycle that involves migrations through several organ systems and development from an infective larvae of 250–300(m in length to an adult worm that can be >25 cm in length (Douvres 1969) (see **figure 1**). After oral uptake of eggs, the newly hatched larvae (both second (L2) and third stage larvae (L3) have been indicated as initiating infection in the pig) penetrate the caecum and proximal colon and migrate via the venous blood to the liver. The peak appearance of early third stage larvae (L3) in the liver is at 4 days post-inoculation (p.i.). This is followed by re-entry into the venous blood and migration to the lungs where they advance to a late L3; peak levels appear in the lungs 7 days p.i. The larvae penetrate into the alveoli, migrate to the trachea and are swallowed gaining access to the small intestine beginning at 10 days p.i. The larvae molt to the fourth stage (L4) shortly after arrival in the jejunum and to the fifth stage or young adult at 23–25 days p.i. Patent egg-laying females shed eggs into the feces by 6–8 weeks p.i. Larval migration results in several clinical signs of infection, including inflammation of the liver marked by intralobular fibrosis and inflammatory cell infiltration that expresses as "milk spots," and petechial hemorrhage in lungs due to an eosinophilic infiltration of the parenchymal tissues. Protective immunity to *A. suum* has been demonstrated by reduced recovery of larvae in the liver and lungs following a re-exposure to infection (Urban 1988; Eriksen 1992; Helwigh 1999). There is also a spontaneous cure of the bulk of larvae in the jejunum between 14 and 21 days p.i. which results in L4 forced to the ileum and later expelled (Roepstorff et al 1997). Chronic exposure of the intestinal mucosa to infective larvae elicits appropriate effector components necessary to prevent larval migration from the intestines (Urban et. al. 1988). There is an accumulation of mucosal mast cells in the intestines of chronically exposed pigs that respond to parasite antigens in vitro by the release of histamine. This could account for a localized intestinal allergic response to infection (Ashraf et. al. 1988). Peripheral blood eosinophilia and the development of specific immune responses from lymph nodes draining the sites of infection are indications of a strong anamnestic response to larval migration. Both enzyme-linked immunospot and immunoabsorbent assays using larval antigens as well as Western blot procedure to evaluate stage-specific *A. suum* antigens have been developed to characterize immunity to this parasite (Jungersen 1999), as well as increases in the numbers of B-cells and CD4+ T cells (Jungersen 2001). However, there is no evidence to suggest that pigs maintain long term sterilizing protection from the acquisition of patent infections.

Recently, the effect of *A. suum* infection on the phenotype and function of cells present in the pulmonary mucosa has been characterized. Changes in cellular composition of the bronchoalveolar lavage (BAL) and associated tracheo-bronquial lymph nodes were evaluated after a primary or secondary infection. The phenotype of BAL cells and the function of alveolar macrophages (AM) were determined by detecting changes in tyrosine phosphorylation assessed by flow cytometry at 7, 11, 14, 28, 32, and 35 days p.i.. A second set of pigs was re-infected after 21 days of initial infection and the BAL evaluated 7, 14, and 21 days later. Our results showed a reduction in the relative numbers of AM in the BAL that corresponds to an increase in the percentage of eosinophils with time

Figure 1. Life cycle of *Ascaris suum*



Life cycle of *Ascaris suum*

Adapted from Murrel K. D., 1986

after inoculation. These changes were greatest at 14 days p.i. The AM made up 98% of BAL in uninfected pigs compared to 44% in infected pigs, while the percentage of eosinophils was 0% and 49%, respectively. Competitive RT-PCR analysis of RNA expression from cells isolated from the draining lymph nodes indicated an increase IL-4 and IL-5 gene expression and no change or a decrease in IFN-(expression. The increase in eosinophils in BAL was also associated with an altered AM function. There was a reduction in phagocytosis of opsonized/formalin-fixed *Staphylococcus aureus* and a decrease in intracellular tyrosine phosphorylation starting at 11 days p.i. It is postulated that *A. suum* infection may affect the production parameters of swine by affecting performance as judged by reducing the ability of AM to become activated and clear secondary microbial infections. These findings could compromise both the innate and acquired immunity to pathogens at the pulmonary mucosa.

Infection of pigs with *A. suum* provides a useful model for the study of local immunity in different organ systems. Furthermore, skewing of the immune response can have negative consequences on the ability of the pig to mount a robust and appropriate response to different classes of pathogens. Identification of parasite antigens or biologically active parasite-derived products that modulate local immunity could lead to the production of better vaccines and greater insight into immune responses that regulate animal health.

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