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Early Pathogenesis in Porcine Proliferative Enteropathy caused by *Lawsonia intracellularis*

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Summary

The intestinal bacterium *Lawsonia intracellularis*, the cause of proliferative enteropathy (PE) in pigs, is believed to infect mitotically active epithelial cells of the intestinal crypts and then multiply and spread in these cells as they divide. Further spread of infection is thought to occur by shedding of bacteria from infected crypts followed by infection of new crypts. The early stages of the pathogenesis of PE, from 0 to 48 hours post-infection (hpi), have not been studied *in vivo*. In the present study pigs were inoculated with *L. intracellularis* and killed from 12 hpi to 5 days post-infection (dpi). The localization of *L. intracellularis* was determined immunohistochemically and by fluorescence in-situ hybridization. At 12 hpi *L. intracellularis* was found within epithelial cells at the tips of villi, indicating infection of a range of epithelial cells including mature differentiated enterocytes. Furthermore, early invasion of the intestinal connective tissue was observed; with the presence of single bacteria in the lamina propria 12 hpi, and with a further spread of bacteria in the lamina propria observed at 5 dpi, suggesting an active role for the lamina propria in the course of infection.

Keywords: infection trial; *Lawsonia intracellularis*; pathogenesis; pig; porcine proliferative enteropathy

Introduction

Infection with the bacterium *Lawsonia intracellularis* is associated with proliferative enteropathy (PE) (McOrist *et al*., 1995a), an important disease of commercial pig production worldwide (Lawson and Gebhart, 2000). The generally accepted pathogenesis of *L. intracellularis*-induced PE involves an initial colonization of the epithelium of the lower part of the small intestine leading to adenomatous thickening of the mucosa, followed by later involvement of the caecum and colon (Lawson and Gebhart, 2000; Guedes and Gebhart, 2004). Microscopically altered epithelium is highly correlated with the presence of intracellular *L. intracellularis* (Rowland and Lawson, 1974; Rowland *et al*., 1975; Joens *et al*., 1993; Lawson and Gebhart, 2000) and the bacterium is hypothesized to infect the mitotically active cells of the intestinal crypts and to be dependent on host cell mitosis for its own propagation (Lawson *et al*., 1993, 1995; Smith and Lawson, 2001). The infection induces proliferation of crypt epithelial cells and inhibits their differentiation into mature enterocytes (Rowland *et al*., 1978; McOrist *et al*., 1989; Lawson and Gebhart, 2000; Smith and Lawson, 2001). Infected cells are exfoliated during their migration to the top of the villus and infected cells are carried downstream with the intestinal mucus, together with free bacteria, and this forms the basis for infection of new crypt epithelial cells (Lawson and Gebhart, 2000; Guedes and Gebhart, 2004).

The initial pathogenesis of *L. intracellularis* infection is to a large extent unexplored. The presence of *L. intracellularis* in the terminal small intestine has been observed at the earliest 3 days post-infection (dpi) in an experimental study *in vivo*, but the significance and role of this finding regarding pathogenesis were not discussed (MacIntyre *et al*., 2003). In another study, infected pigs were killed as early as 24 hours post-infection (hpi). Infection was not found

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at 24 hpi and 3 dpi, but low-level infection of the small intestine was seen at 5 dpi (Guedes and Gebhart, 2004). In contrast, in-vitro laboratory data have shown uptake of bacteria by cultured cells as early as 3 hpi (McOrist *et al*., 1995b).

The aim of the present study was to characterize the early stage of infection in porcine PE. The specific objectives were to characterize target areas and cells of the intestinal tract and to define the development of lesions and spread of *L. intracellularis* during the early phase of infection. Two infection trials were conducted and the animals were killed between 12 hpi and 5 dpi. As it is not possible to grow a pathogenic strain of *L. intracellularis* in cell culture, an infection model using mucosal scraping from the small intestine of naturally diseased pigs was used for challenge, a model that previously has been used successfully by others (Guedes and Gebhart, 2003; Boesen *et al*., 2004; Boesen *et al*., 2005b).

A polyclonal rabbit anti-*L. intracellularis* antibody (PAb) was produced and characterized prior to the infection trials. The antibody was used to detect *L. intracellularis* bacteria in intestinal tissue by immunohistochemistry (IHC). In addition, fluorescence in-situ hybridization (FISH) with a specific oligonucleotide probe targeting the 16S ribosomal RNA (rRNA) of *L. intracellularis* was performed (Boye *et al*., 1998). These two techniques are complementary and allow differentiation between live and dead bacteria. While IHC recognizes antigenic structures of viable and dead bacteria (including phagocytosed and disintegrated bacteria), oligonucleotide probes bind to rRNA in viable bacteria with an intact cell membrane. Although oligonucleotide probes may also bind to DNA, this is present in so few copies per cell that ISH principally detects rRNA, which is abundant in metabolically active bacteria.

Materials and Methods

Infection Trials

Two trials were performed. In trial 1, pairs of animals were killed at 48 hpi and 5 dpi and in trial 2, pairs of animals were killed at 12, 24, 36 and 48 hpi. Trial 1 served as a pilot study to evaluate the extent to which the early phase of infection could be detected. The procedures in the trials were essentially the same, but the sampling of tissues varied. In trial 1 the following samples were taken: stomach, lower jejunum, ileum, caecum, colon, ileocaecal lymph node, lung, liver, spleen and kidney. The sampling was extended in trial 2 to also include duodenum and six evenly distributed areas of jejunum and rectum. Each intestinal sample comprised a 5 cm length of intestine.

In both trials, 5–6-week-old piglets were obtained from a 'high health' farm considered to be free of *L. intracellularis* infection after a medical eradication program. Absence of *L. intracellularis* infection was confirmed by performing an enzyme linked immunosorbent assay (ELISA) on blood samples and by routine polymerase chain reaction (PCR) analysis of faecal samples (Lindecrona *et al*., 2002). The piglets were housed together indoors at the National Veterinary Institute, Technical University of Denmark. Box temperatures in both trials were held at 24°C and natural daylight periods were used. The pigs were fed a standard commercial pelleted diet *ad libitum* (DLG +10; Aarhus, Denmark) and had free access to water from the city water supply system. The piglets were inoculated 2 weeks after arrival at the National Veterinary Institute. Piglets were fasted for 24 h prior to inoculation with 30 ml of mucosal scraping delivered by stomach tube. After inoculation, the piglets in trial 2 were immediately fed sesame seeds so that movement of the stomach contents through the intestinal tract could be determined at the time of necropsy examination. Animals were killed by bolt pistol followed exsanguination. The experiments were conducted in accordance with the guidelines laid down by the Danish Animal Experiments Inspectorate under the Ministry of Justice.

In trial 1, two control piglets were given a mock inoculum of vehicle (sucrose–potassium–glutamate, pH 7.0) alone and were housed separately. These animals were killed at 5 dpi. In trial 2, two uninoculated piglets comprised the negative controls. All animals were subject to necropsy examination where tissue samples were collected and fixed in 4% neutral buffered paraformaldehyde for 24 h. Three full transverse sections were cut from each sample of small intestine and one transverse section was taken from the colon and caecum. The sections were processed routinely, embedded in paraffin wax and sectioned (5 μm). All tissue slides were evaluated using IHC and selected slides were evaluated by FISH.

The mucosal scraping that formed the inoculum was obtained from the intestine of pigs with PE from a single affected farm. Sections of small intestines with significant gross changes consistent with PE were sampled and pieces from both ends of each sampled section were subject to IHC (Jensen *et al*., 1997) in order to confirm the aetiology and ensure the presence of high numbers of *L. intracellularis* bacteria in affected epithelium. Pigs from this farm had previously been successfully employed for the induction of experimental infection (Boesen *et al*., 2004). The intestines were frozen at −80°C until used. The intestines were thawed and the mucosa was scraped using a sterile scalpel. The mucosal scrapings were added to