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Immunological responses to vaccination following experimental *Lawsonia intracellularis* virulent challenge in pigs

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ABSTRACT

Although a live attenuated vaccine has been used extensively to provide immunity against porcine proliferative enteropathy (PE) caused by *Lawsonia intracellularis*, the nature of the protective response is an area of considerable interest for the control of PE. Two trials investigated immune responses in pigs after oral and intramuscular (IM) vaccination followed by virulent *L. intracellularis* challenge. After an oral vaccination with $10^{5.9}$ $TCID_{50}$ organisms, significantly increased serum and mucosal secretions of IgM, IgG and higher mucosal TNF-α and TGF-β1 were detected by day 17, together with a trend towards higher levels of IFN-γ and IL-6. Pigs vaccinated IM produced elevated serum antibody titres but mucosal immune responses were not detected. After challenge with virulent *L. intracellularis*, non-vaccinated control pigs had higher PE lesion scores and excreted significantly higher numbers of *L. intracellularis* in faeces than the vaccinated pigs. Reduced intestinal pathology and faecal *L. intracellularis* shedding were evident in the vaccinated groups. The results indicated that protection was associated with mucosal cytokine and specific IgG and IgA responses after vaccination and that systemic antibody responses were boosted following challenge. However in the search for an immune correlate with protection, a causal association was not evident from a kinetic analysis of immune parameters in serum, ileal pathology and faecal shedding.

1. Introduction

The Gram-negative intracellular bacterium *Lawsonia intracellularis* causes proliferative enteropathy (PE), characterised by diarrhoea and poor performance in growing pigs and severe haemorrhagic diarrhoea in finisher and breeding animals (Lawson and Gebhart, 2000). The most consistent macroscopic pathology of PE is thickening of the intestinal mucosa which is associated histologically with proliferation of immature enterocytes (Lawson and Gebhart, 2000). The clinical signs and lesions can be controlled using a commercial oral live attenuated vaccine (Enterisol® Ileitis, Boehringer Ingelheim) (Kroll et al., 2004; McOrist and Smits, 2007). This vaccine contains an attenuated *L. intracellularis* isolate (B3903; $10^{4.9}$ $TCID_{50}$/dose) originally isolated from the ileum of a Danish pig with acute proliferative haemorrhagic enteropathy (PHE) (Kroll et al., 2004). Protective immunity against re-infection is also apparent in recovered pigs (Collins and Love, 2007; Cordes et al., 2012; Riber et al., 2011b).

A conventional immunological approach to induce mucosal immunity against gut pathogens has involved oral vaccination or intraperitoneal inoculation (Muir et al., 1998). However, where antibody provides mucosal protection, high levels of serum IgG engendered by systemic

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vaccination have been effective against some intracellular bacteria. For example, *Salmonella enterica* serovar Typhimurium bacterin administrated intramuscularly reduced lesions and shedding in naturally infected pigs (Farzan and Friendship, 2010). Similarly, an intramuscular *L. intracellularis* killed bacterin induced significant protection to PE after virulent challenge (Dale et al., 1997), and in a patent description, protection was induced after immunisation with killed *L. intracellularis* (Jacobs et al., 2011).

To identify immune responses which might indicate the successful induction of protective immunity following vaccination with Enterisol® Ileitis this study measured local mucosal and systemic immune responses in the first 3 weeks after immunisation. Since local mucosal responses were marginal following a conventional single dose vaccination in a preliminary trial, pigs were vaccinated orally with a ten times dose (10×) of Enterisol® Ileitis. Local mucosal and systemic antibody and selected cytokine responses were measured and compared with those from piglets given the same dose of vaccine intramuscularly (IM). To determine whether these responses could foreshadow protection, the remaining vaccinated cohorts were challenged and immunity assessed by serological assays, reduction of clinical signs and intestinal lesions and the duration and magnitude of bacterial shedding in faeces.

2. Materials and methods

2.1. Animal ethics

All animal experiments were performed according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and approved by the University of Sydney and Elizabeth MacArthur Agricultural Institute Animal Ethics Committees, Australia.

2.2. Experimental design

For trials 1 and 2, a total of 40 and 62 Landrace × Large White pigs, respectively, aged 3–4 weeks, were purchased from a commercial herd clinically and serologically free of PE. Piglets were transferred to a controlled environment research facility, with access to water and fed on weaner/grower diet (Vella Stock Feeds, Australia) with no medication. Pigs were weighed (6.0 ± 0.5 kg) and randomly allocated into respective treatment groups housed in separate pens under strict quarantine conditions. Pigs were monitored daily for body condition, clinical signs and abnormal behaviour.

2.3. Lawsonia vaccination

Prior to vaccination (d0) at 5 weeks of age, pigs were bled by jugular venipuncture and faeces were collected from individual pigs to confirm the absence of *L. intracellularis* or the presence of maternal antibodies that might interfere with vaccination. The lyophilised vaccine (Enterisol® Ileitis, Boehringer Ingelheim Pty Ltd., SA-122A-323) was reconstituted according to the manufacturer's instructions to either standard (1×) dose ($10^{4.9}$ $TCID_{50}$) or ten times ($10^{5.9}$ $TCID_{50}$) concentrations. Pigs received the appropriate vaccine dose orally in 2.0 mL diluent by drenching gun or by intramuscular inoculation (IM) in the right deltoid muscle (cervical area). Negative and positive control pigs were not vaccinated.

2.4. Single dose vaccination trial

Forty pigs were allocated into three treatments, where group 1 ($n = 16$) received 1× oral vaccine, group 2 ($n = 16$) received 1× IM inoculation and group 3 pigs ($n = 8$) were not vaccinated. Four pigs from each of groups 1 and 2 and two pigs from group 3 were necropsied sequentially on each of days 7, 14, 21 and 28 after vaccination. On each of these days, the remaining pigs were bled for serum. At necropsy, the left and right pre-scapular lymph nodes were weighed and blood taken from the jugular vein. Ileal and jejunal tissue was sampled at 10 cm and 1 m from the proximal ileo-caecal valve (ICV), respectively, together with adjacent mesenteric lymph nodes (MLN). Each tissue and MLN sample (ca 2 mm × 2 mm) was stored in 10% neutral-buffered formalin fixative until analysis. In the adjacent segment, the intestinal mucosal secretions were collected by gently scraping the ileal mucosa with a sterile scalpel and placed into 2 ml phosphate-buffered saline (PBS, pH 7.2) containing 0.2 M EDTA on ice. The scrapings were each stored at −20 °C. Prior to assay, mucosal scraping samples were thawed and adjusted to a protein concentration 25 mg of protein ml^{-1}.

2.5. Ten times vaccination trial with challenge infection

2.5.1. Experiment 1: Analysis of immune responses after vaccination

Fifteen pigs aged 4 weeks were randomly assigned into three treatment groups: six pigs in each of groups 1 and 2 were orally vaccinated and IM vaccinated, respectively, with 10× dose of Enterisol® Ileitis vaccine and three pigs in group 3 were not vaccinated. Sera were obtained from each pig by venipuncture of the jugular vein before vaccination and necropsy. Seven pigs were randomly selected (3 from groups 1 and 2, and one from group 3) and euthanized on d9 post vaccination, and the remainder on d17. At necropsy, pre scapular lymph node weights, ileum sections and mucosa secretions were collected as described above for the single dose trial.

2.5.2. Experiment 2: Immune responses after a challenge infection

Forty-six pigs were randomly allocated into 5 treatment groups. Groups 1–3 (10 pigs each) were vaccinated with 1× oral (1xOR – group 1), 1× IM (1xIM – group 2), 10× oral (10xOR – group 3), respectively, while 10 positive control pigs (PC – group 4) remained unvaccinated. Vaccination was carried out simultaneously with the 15 pigs used in Experiment 1 above. Six pigs were kept unvaccinated and unchallenged as negative controls (NC – group 5). Four weeks after vaccination, each pig in groups 1–4 was dosed orally with *L. intracellularis* infected intestinal mucosa inoculum prepared and quantified as described elsewhere (Collins and Love, 2007; Collins et al., 1996). Briefly, pigs