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Effect of the route of administration on the mucosal and systemic immune responses to *Lawsonia intracellularis* vaccine in pigs

MG Nogueira,[a*] AM Collins,[b] RH Dunlop[c] and D Emery[a]

In an on-farm study, 40 weaned piglets aged 3 weeks were vaccinated with *Lawsonia intracellularis* vaccine orally, IM or IP while a fourth group remained unvaccinated. All vaccinated animals showed increased serum levels of *L. intracellularis*-specific IgG antibodies, but significantly elevated concentrations of specific IgG, IgA and cytokines were generated in ileal mucosal secretions from the orally and IP vaccinated pigs when examined at 17 days after vaccination.

Keywords immunity; *Lawsonia intracellularis*; pigs; vaccination

Abbreviations IFN, interferon; IL, interleukin; PE, porcine enteropathy; pv, post vaccination; TGF, transforming growth factor; TNF, tumour necrosis factor

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Lawsonia intracellularis is an obligate intracellular gram-negative bacterium that causes porcine enteropathy (PE),[1] an economically significant production problem that reduces profitability and requires the use of a vaccine or inclusion of antibiotics in the feed or water of grower and finisher pigs to prevent outbreaks on farms. Earlier studies have demonstrated humoral and cell-mediated immune responses after natural and experimental exposure with pathogenic *L.* intracellularis,[2,3] and oral administration of live attenuated *L. intracellularis* vaccine reduces the clinical signs and lesions of PE.[4] However, a specific, quantifiable immune response/marker that consistently indicates protection has not been defined. Oral delivery of mucosal vaccines reliably induces immunity against enteric pathogens, but protection has also been demonstrated after IP or IM inoculation.[5]

In an earlier experimental challenge trial, we successfully induced protection using IM vaccination against experimental *L. intracellularis* challenge.[5] Potential immune correlates for protection were identified, but their specificity in the field needed to be tested. At the same time as *L. intracellularis* vaccination, weaner pigs face multiple challenges that stimulate the immune system, including the introduction of solid feed and infections with other pathogens such as haemolytic *Escherichia coli*. Therefore, strategies to efficiently induce protective mucosal immunity to a range of enteric diseases in young pigs are necessary, especially to complement nutritional additives and to replace the need for antibiotics in feed. To ensure that vaccines have effectively immunised animals prior to pathogen exposure, knowledge of the immune response is needed. Therefore, we compared local and systemic immune responses after oral, IM and IP inoculation of *L. intracellularis* vaccine in an on-farm trial.

*Corresponding author: mariana_nogueira@hotmail.com.
[a]Farm Animal and Veterinary Public Health, University of Sydney, Camden, New South Wales, Australia
[b]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Narellan, New South Wales, Australia
[c]Chris Richards and Associates Pty Ltd, East Bendigo, Victoria, Australia

Materials and methods

This study was approved by the animal ethics committees at Elizabeth Macarthur Agricultural Institute and the University of Sydney (AEC-M12/03). The trial was performed in a commercial piggery in Victoria, Australia, with all-in/all-out production flows of Landrace/Large White crossbred pigs and grow-out sites.

Gilts and sows were routinely vaccinated against pathogenic neonatal *E. coli*, *Haemophilus parasuis* and *Streptococcus suis* at introduction to the herd and 3 weeks prior to farrowing. Gilts and sows are also vaccinated against erysipelas, porcine parvovirus and leptospirosis at weaning. During the past decade, the farm had had only one case of respiratory disease, possibly associated with *Mycoplasma hyopneumoniae* or *Actinobacillus pleuropneumoniae*. Pre- and post-weaning mortality rates were less than 10% and 3.5%, respectively. Piglets were routinely weaned at 22–24 days of age and moved into an all-in/all-out room until 8 weeks of age, then moved into a naturally ventilated grower facility until 16 weeks of age. For this experiment, most of the routine farm practices were kept for sows and piglets. Antibiotics (macrolides) were removed from feed and water 3 days before and after vaccination.

At 5 days pre-weaning (17–19 days of age), the pigs were grouped by randomly selecting four offspring from one sow and allocating one piglet to each of four treatment groups. This was repeated for 10 sows until each group had 10 piglets. At 26 days of age (just after weaning), 10 piglets were orally vaccinated with 2 mL of ×10 dose concentrate containing $10^{5.9}$ $TCID_{50}$ attenuated *L. intracellularis* (Enterisol® Ileitis, Boehringer Ingelheim Vetmedica, MO, USA); a second group was given the same dose IM and the third group was vaccinated with the same dose IP (day 0). The remaining 10 piglets were kept unvaccinated as a negative control group.

Serological responses were measured on days 0, 8 and 17 post vaccination (pv) and ileal scrapings were collected at necropsy on day 17, as previously described.[6] Blood and mucosal secretions were assessed for *L. intracellularis*-specific IgG using bioScreen® Ileitis Antigen ELISA test (Synbiotics, MO, USA) and mucosal IgA using an experimental direct ELISA as detailed previously.[6] The quantities of cytokines, interferon gamma (IFN-γ), interleukin (IL)-6, IL-10,

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Table 1. Mean and standard error of mean (SEM) for *Lawsonia intracellularis* antibody and cytokine immune responses in porcine ileal mucosal secretions at day 17 post vaccination

Treatment	Antibodies in mucosal secretions		Cytokines in mucosal secretions				
	IgG (PI value)	IgA (titre)	IFN-γ (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)	TGF-β1 (pg/mL)
Oral	43.7*	24.7*	15.7	60.4	28.1	83.2*	285.3*
IM	39.5*	10.3	12.7	23.5	19.8	8.8	3.1
IP	44.2*	28.3*	10.2	64.3	16.2	30.5*	174.4*
Unvaccinated	28.6	7.6	9.5	20.0	14.6	0.2	0.5
SEM	4.4	2.6	2.0	17.5	7.6	13.0	53.0
P (<0.05)	0.031	<0.001	0.162	0.059	0.605	<0.001	<0.001

*Statistically significant differences (P < 0.05) between the respective vaccinated group and the unvaccinated controls in each column.
IFN, interferon; IL, interleukin; PI, percentage inhibition; TGF, transforming growth factor; TNF, tumour necrosis factor.

tumour necrosis factor alpha (TNF-α) and transforming growth factor beta-1 (TGF-β1), in ileal secretions were determined with porcine Quantikine ELISA assay kits (R&D Systems, MN, USA). Statistical analysis was performed using the restricted maximum likelihood test (GenStat Release 13th edn, Oxford, UK).

Results and Discussion

This study demonstrated that systemic/humoral and local mucosal immune responses after IP inoculation of the *L. intracellularis* vaccine paralleled those induced by oral vaccination. The *L. intracellularis*-specific IgG reactivity in serum increased ($P < 0.05$) significantly from days 0 to 17 pv in each of the three vaccinated groups.

Serum immune responses were below the limit of detection (1%) before vaccination, but by day 17 pv the pigs in the oral (28.9 ± 1.9%), IP (11.7 ± 1.9%) and IM (15.8 ± 1.5%) vaccinated groups had produced a significant ($P < 0.05$) increase in *L. intracellularis*-specific IgG when compared with the unvaccinated animals (3.7 ± 0.9%). Oral and IP vaccination also generated significant ($P < 0.05$) increases in *L. intracellularis*-specific mucosal IgG and IgA, whereas the response to IM vaccination was limited to increased IgG (Table 1).

Oral dosing with live attenuated vaccine induces a protective immune response at mucosal surfaces, which blocks pathogen attachment or neutralises local virulence factors by specific IgA or IgG antibody, but IP immunisation is equally efficacious.[2,4] In the present study, oral and IP vaccination consistently elicited the highest mucosal responses (Table 1). It was anticipated from previous reports that the serosal surface of the intestine and draining mesenteric lymph nodes may be stimulated by IP delivery,[5,7,8] especially if accompanied by inflammatory adjuvant or bacterial components, and would induce mucosal immunity with secretion of bioactive products into the intestinal lumen. Previously, IP vaccination with adjuvant protected pigs from lung lesions caused by *M. hyopneumoniae* or *A. pleuropneumoniae* infection.[9,10]

In this on-farm study, the ileal antibody and cytokine responses following IP immunisation resembled those induced by the oral, rather than IM, route of vaccination (Table 1). The quantities of the inflammatory cytokines IFN-γ and IL-6 (16 and 60 pg/mL, respectively) in the ileal secretions following oral vaccination were less than those in previous pen trials (750 and 225 pg/mL, respectively),[6] whereas the concentrations of TNF-α and TGF-β1 were comparable. In the current study, there were significantly ($P < 0.05$) increased mucosal concentrations of TNF-α and TGF-β1 after oral and IP immunisation (Table 1), as well as increased IL-6 ($P = 0.059$). Although the protein concentrations of mucosal secretions were standardised between studies, and the pigs were healthy and without signs of diarrhoea, the range of mucosal responses of individual pigs can vary substantially in on-farm trials.[9] Compared with the higher quantities of cytokines reported previously,[5] the concentrations of IFN-γ, IL-6 and TNF-α in the present trial may reflect the use of antibiotics, the different gastrointestinal microflora in the piglets or the timing of sampling, but the aim was to highlight the response to *L. intracellularis*. Previously, *L. intracellularis*-specific IFN-γ-producing cells have been detected by ELISPOT in porcine peripheral blood mononuclear cells at 4–13 weeks pv.[3] That method is more sensitive than analysis of mucosal secretions because it measures the response of primed T-lymphocytes after a second exposure to *L. intracellularis* antigen in vitro.[6] The specificity of the cytokine response to vaccination in the present trial was also supported by the higher concentrations of TGF-β1, possibly related to local mucosal repair and immune regulation resulting from the limited infection associated with vaccine "take". However, the comparable spectrum of immune responses generated after vaccination, and the subsequent protection after challenge in the previous pen trial,[6] suggests that significant protection against *L. intracellularis* could be anticipated in each of the vaccinated groups in this study. Most importantly for mucosal protection, IgA was generated by oral and IP vaccination. The results from both of our studies indicate that if a specific antibody response can be detected following vaccination, a protective level of immunity may have been induced against challenge.

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