Vaccine 36 (2018) 1500–1508

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A novel inactivated vaccine against *Lawsonia intracellularis* induces rapid induction of humoral immunity, reduction of bacterial shedding and provides robust gut barrier function

F. Roerink [a,*], C.L. Morgan [a], S.M. Knetter [a], M.-H. Passat [b], A.L. Archibald [b], T. Ait-Ali [b,*], E.L. Strait [a]

[a] Merck Animal Health, 2 Giralda Farms, Madison, NJ 07940, USA
[b] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Roslin, Midlothian EH25 9RG, UK

ARTICLE INFO

Article history:
Received 11 September 2017
Received in revised form 8 December 2017
Accepted 18 December 2017
Available online 11 January 2018

Keywords:
Swine
Ileitis
Lawsonia intracellularis
Intestinal integrity
Vaccine
Efficacy

ABSTRACT

Porcine proliferative ileitis is a major economic burden for the swine industry, affecting growing pigs and young adult pigs. In this study, the protective efficacy of an inactivated, injectable whole-cell bacteria vaccine against *L. intracellularis* – Porcilis® Ileitis was evaluated under field conditions.

Eighty-five, three-week-old pigs on a commercial farrow-to-finish farm were vaccinated by the intramuscular route, either with a dose of injectable vaccine, or with saline. A subset of vaccinates and control pigs were necropsied at 21 days post-challenge. Incidence and severity of ileitis were evaluated by gross and microscopic observation of ileal tissues. Colonization of the gut after challenge was examined by *L. intracellularis*-specific immunohistochemistry, and qPCR of ileal scrapings. Integrity of the intestinal barrier was evaluated to quantify a range of intestinal markers including secreted mucin and intestinal alkaline phosphatase, and innate immune markers including Caspase-3 and Calprotectin. A second subset of pigs was monitored for fecal shedding of *L. intracellularis*, until resolution of shedding.

Our investigation indicated that Porcilis Ileitis provided robust protection against ileitis, reduced bacterial shedding 15-fold ($p < .05$) and preserved normal gut barrier function in the face of an experimental challenge with virulent *L. intracellularis*.

1. Introduction

Ileitis caused by *Lawsonia intracellularis* (*L. intracellularis*) continues to be a problem in swine production systems worldwide. *L. intracellularis* is a Gram-negative, intracellular bacterium that can infect a number of animal species, but it is of particular economic concern in the swine industry. In pigs, the bacteria cause porcine proliferative enteropathy (ileitis). Clinically affected animals exhibit diarrhea and reduced growth performance, resulting in increased time to market and greater variation in size between pigs. In young adults, the infection can lead to an acute hemorrhagic form of the disease, characterized by dark, tarry diarrhea and which may result in death. *L. intracellularis* also infects pigs sub-clinically, without clear clinical signs but still resulting in reduced growth performance. Its worldwide distribution and high prevalence have been recognized since the initial characterization of this pathogen in the early 1990s and *L. intracellularis* is reported to affect 57–100% of herds, globally [1–3].

As an obligate intracellular pathogen, interaction between *L. intracellularis* and host cells is crucial in establishing infection. The bacterium infects the gastrointestinal tract, with a specific tropism for the terminal ileum. The hallmark lesion of *L. intracellularis* infection is the proliferation of intestinal crypt lining cells (enterocytes) which results in hyperplasia of the mucosal wall. The peak of bacterial burden is associated not only with crypt epithelial cell proliferation but also with down-regulation of specific host mechanisms involved in cell transport and maintenance of mucosal integrity, and with inflammation [4–6]. It is likely that the poor performance and growth of affected animals are a direct consequence of these cell differentiation alterations [7].

There are some tools available for controlling *L. intracellularis* infections and limiting the associated economic losses. Infection by the bacterium can be treated with various antibiotics, notably those from the macrolide, pleuromutilin, and quinoxaline groups [7]. For prophylaxis, a modified live-attenuated vaccine has been commercially available since 2001 [8]. Due to the live nature of the oral vaccine, concurrent use with antibiotics effective against

* Corresponding authors.
E-mail addresses: frank.roerink@merck.com (F. Roerink), tahar.aitali@roslin.ed.ac.uk (T. Ait-Ali).

https://doi.org/10.1016/j.vaccine.2017.12.049
0264-410X/

L. intracellularis is not possible. The use of the oral vaccine requires strict management practices to avoid the simultaneous use of antibiotic treatments. However, prophylactic use of an inactivated vaccine would not be limited in this way.

In this study, the effectiveness of a novel inactivated injectable vaccine, Porcilis Ileitis, as an aid in the control of ileitis caused by *L. intracellularis* was examined. This vaccine was administered to three-week-old pigs under typical field conditions, without restricting the use of antibiotics. Our investigation indicates that Porcilis Ileitis vaccine can provide robust protection against ileitis, help reduce bacterial shedding 15-fold ($p < .01$), and help maintain gut barrier function integrity.

2. Materials and methods

2.1. Ethical statement

The animal trial was conducted by Swine Services Unlimited (Rice, MN, USA, SSUI) as a randomized, blinded study, approved by the SSUI Institutional Animal Care and Use Committee.

2.2. Vaccine

The vaccine used in this study contained inactivated *L. intracellularis* bacteria in XSOLVE adjuvant (Porcilis Ileitis, serial 02381108, Merck Animal Health, Madison, NJ USA). The vaccine is an oil-in-water emulsion. The adjuvant is based on both mineral oil and alpha tocopherol (Vitamin E).

2.3. Study design

Eighty-five mixed-breed and mixed-sex pigs were enrolled on a commercial, farrow-to-finish farm, which farrowed 30 litters per week, weaning 300 pigs per week. The herd was health-stable prior to and during the study, with historic pre-weaning mortality rates between 8 and 10% during the previous 2 years, and nursery mortality of approximately 3%. The herd did not show clinical signs of *L. intracellularis* infection, and all study pigs were negative for anti-*L. intracellularis* antibodies as measured by a commercial inhibition ELISA (bioScreen Ileitis Antibody ELISA, Svanova, Sweden).

The study design is summarized in Fig. 1. Pigs were allocated to the treatments using a random number generator, so that both vaccinates and placebo injected controls were represented within all litters. At 22–25 days of age (23 days median), 40 pigs were given a single 2 mL vaccination intramuscularly in the neck, using a 20 gauge, ¾ in. needle. Another 40 pigs were injected with 2 mL of normal saline as a control. Five pigs were allocated to a sentinel group, and these pigs were administered normal saline in the same manner as the control pigs.

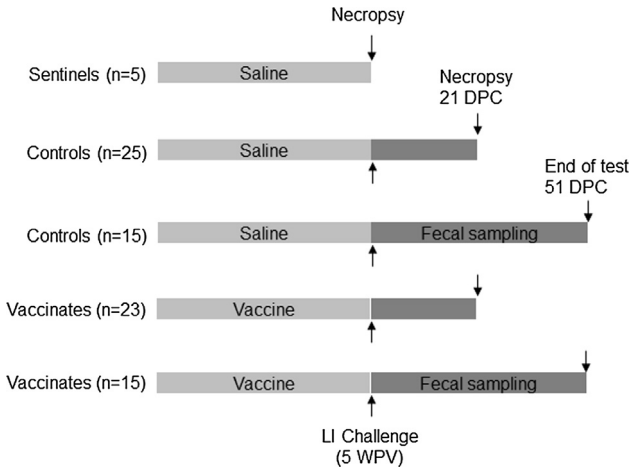

Fig. 1. Study design. Read this figure in conjunction with supplementary Table 1 and the materials and methods section.

The pigs were weaned one day after their vaccination. Upon weaning, the pigs were transported to isolation facilities for the pre-challenge and challenge phases of the study. Pigs from each group were comingled with approximately equal numbers of vaccinates and controls in each pen.

Prior to the challenge, the treatment groups were divided randomly into a subset of 25 pigs to be necropsied at 21 days post-challenge (dpc) (necropsy groups), and a subset of 15 pigs to be fecal sampled to determine *L. intracellularis* shedding during the post-challenge period (sampling groups, see Fig. 1). Randomizations were performed using the Microsoft Excel function Rand() [9]. Challenge occurred five weeks post-vaccination, when the pigs were approximately 8 weeks of age. The timing of necropsy was chosen based on published results of experimental infections [7,10] and coincided with the peak time of fecal shedding and ileal lesions found in preliminary experiments with this challenge model (data not shown).

Prior to the start of the study, as well as prior to the challenge, all pigs were bled and sera were prepared. Pigs were bled again at the end of the test, at 21 dpc for the necropsy groups and at 51 dpc for the shedding groups.

2.4. Husbandry

Water was provided *ad libitum* to all animals. Feed met or exceeded the minimum nutritional requirements for animals of this age and complied with standard procedures for the site. During the pre-weaning phase, the piglets had access to the dam's feed and water dispensers. Sow feed during this phase was a standard, non-medicated lactation diet. Feed was medicated with Mecadox (carbadox) at 50 g/ton, from the day after the vaccination when the pigs were weaned, until two weeks later (14 dpv). Carbadox is a quinoxaline antibiotic indicated for the control of swine dysentery and control of bacterial swine enteritis caused by *Salmonella choleraesuis*, but also effective against *L. intracellularis*, with a reported intracellular minimum inhibitory concentration of <0.5 μg/mL [11,12]. Three weeks after weaning, mild loose stools were observed in some of the pens. All pigs were therefore treated for three days with gentamicin between 22 and 24 dpv. This medication was administered through the water supply, at 1 mg/pig/day. Two pigs were removed from the study at 9 and 12 dpv due to head-tilt and ataxia. Both pigs were allocated in the vaccinated necropsy group. Diagnostic work-ups resulted in diagnoses of bacterial meningitis and possible otitis media, considered unrelated to the vaccination.

2.5. Challenge

2.5.1. Challenge material

At the time of challenge, the five sentinel pigs were necropsied and evaluated for evidence of *L. intracellularis* exposure as detailed in Section 2.8 Necropsy. The remaining 80 pigs were individually orally challenged by syringe into the back of the mouth with 25 mL of *L. intracellularis*-infected gut homogenate, containing approximately 9.3 Log_{10} of *L. intracellularis*, as determined by qPCR. The gut homogenate was prepared from intestines of pigs experimentally challenged with a virulent isolate obtained in 2008 from pigs in the United States displaying clinical signs of ileitis. Briefly, mucosa from guts of pigs with clinical signs of ileitis were collected by scraping, diluted in sucrose-phosphate-glutamate buffer,