Comparative Immunology, Microbiology and Infectious Diseases 36 (2013) 489–498

Contents lists available at SciVerse ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

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

Evaluation of protection conferred by a *Salmonella* Typhimurium inactivated vaccine in *Salmonella*-infected finishing pig farms

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ARTICLE INFO

Article history:
Received 10 October 2012
Received in revised form 16 April 2013
Accepted 2 May 2013

Keywords:
Salmonella
Vaccine
Control
Farm
Typhimurium
Pig

ABSTRACT

The efficacy of an inactivated *S.* Typhimurium vaccine administered to pigs at the beginning of the fattening period was evaluated in four clinical trials (trials A, B, C and D). Faecal shedding and the systemic antibody response during fattening, as well as, the cecal contents and mesenteric lymph nodes collected after slaughtering were used to assess the outcome. *Salmonella* shedders prevalence in the control groups was six times higher than in the treated groups in trials A and D, both herds infected by *S.* Typhimurium. The risk of positive pens was also four or five times higher for the pens housing control pigs in trials A and C. Lower prevalence of *Salmonella* was observed in the slaughter samples from the vaccinated pigs in trial D and in the cecal content samples in trial A, when just the *S.* Typhimurium results were compared. The results suggest the effective homologous protection of the vaccinated pigs; however, the high humoral response elicited in the vaccinated pigs complicates their use in farms under serological surveillance programmes.

1. Introduction

Bacterial foodborne pathogens are of increasing concern worldwide. The large number of human cases of *Salmonella* infection and outbreaks of the pathogen reported each year [1], together with the development of antimicrobial resistance [2], make *Salmonella* one of the most relevant of the foodborne pathogens. Pork and pork products are common sources of human salmonellosis [1,3], and in a number of countries, the occurrence of *Salmonella* is monitored in the pork production industry [4–6]. Currently, these control programmes are not compulsory in the EU; however, Regulation 2160/2003 has established the need for developing appropriate and effective measures to detect and control *Salmonella* at all relevant stages of the pork production chain, particularly at the primary production level [7]. Although *Salmonella* infection can occur at all phases of swine production, the finishing stage is particularly relevant because finishing pigs usually become infected during fattening [8,9]. Moreover, these infected market-weight pigs continuously introduce *Salmonella* into the slaughterhouse environment [10,11]. Therefore, it is believed that the reduction of *Salmonella* prevalence at this stage in the production chain will contribute significantly to protecting human health [12]. The reduction in *Salmonella* prevalence in swine farms can be achieved through several measures, including vaccination, sanitation, medication, and the management of known risk factors.

The pig-to-pig transmission of the pathogen usually occurs via the faecal–oral route, and self-limiting enterocolitis is the most common clinical sign of infection in pigs (obviating *S.* Cholerasuis). However, *Salmonella* infections often pass subclinically in swine, seldom exhibit clinical

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http://dx.doi.org/10.1016/j.cimid.2013.05.002

symptoms, but intermittently shed the bacteriain the faeces. Infected pigs may develop as carriers of *Salmonella* in several organs and tissues, particularly in the tonsils and mesenteric lymph nodes. These carrier pigs are a major source of contamination for other animals, the environment, or even the carcasses in the slaughterhouse [13].

Swine can be infected with a wide variety of *Salmonella enterica* serovars. *S.* Typhimurium is of particular interest because this serotypes the most prevalent in swine [14–16] and the second most frequent in human infections [1], frequently related to pork consumption in the EU [17]. Moreover, *S.* Typhimurium is often linked to human outbreaks caused by clones, such as the DT104 [18,19], which frequently harbours antimicrobial resistance genes [20], thus limiting the therapeutic alternatives [21].

Several studies have demonstrated that vaccination is an alternative for the control of *Salmonella* during swine production, although they used different vaccination protocols and evaluation parameters [22–25]. Despite the fact that live vaccines elicit better protection [26], several studies have used inactivated vaccines with promising results [23]. The present study aimed at investigating the efficacy of immunising fattening pigs with an *S.* Typhimurium inactivated vaccine to prevent and/or reduce the infection at the farm level and, consequently, the risk of delivering infected pigs to the slaughterhouse.

2. Materials and methods

2.1. Experimental design

The control blinded clinical trials (trials A, B, C and D) were performed at four different fattening units with all-in/all-out management. Of the fattening units, two were part of farrow-to-finish farms (units A and B), and two were part of three-site vertical integration systems (units C and D). The pigs in units A, B and D came from a unique breeding farm, whereas the pigs in unit C were from two different origins. Information regarding the number of pigs bred per farm and housed per barn and pen is indicated in Table 1, which includes the data regarding the fattening duration and outcome reported.

The farms were selected to participate in this study based on historical data of persistent *Salmonella* infection and on the results of the previous fattening period, when the infection was corroborated by serology (50 pigs were tested) and bacteriology (10 faecal pooled pen samples were tested) (Table 1). The absence of other pathogens that frequently cause gastrointestinal disorders at fattening (*Brachyspira hyodysenteriae*, *Brachyspira pilosicoli* and *Lawsonia intracellularis*) was also confirmed. Moreover, after routine cleaning and disinfection protocols and prior to the entrance of the pigs, samples from the pens and corridors and dust from the farm environment were checked for the presence of *Salmonella*.

As shown in Fig. 1, two different approaches were followed for the group distribution and sample collection. Experiment 1 (trials A, B and C) was performed in two fattening units containing two barns that were filled simultaneously (units A and C) and one fattening unit containing just one facility (unit B). Once all the pigs were housed,

Table 1
Main characteristics of the four *Salmonella* infected finishing herds selected for the evaluation of the protection conferred by a *Salmonella* Typhimurium-inactivated vaccine.

Clinical trial	Type of farm	No. pigs per farm	No. barns (pigs per barn)	No. pigs per pen	Randomisation	Age of vaccination (approx)	Vaccine boost (days later)	Fattening duration (final weight)	Outcome before vaccination	
									Seroprev.[a]	Serotype
A	Farrow to Finish	420	2 (180/240)	15	By pen	81 days of life	25	95 days (100 kg)	44%	Typhimurium
B	Farrow to Finish	400	1	15	By pen	68–75 days of life	21	90 days (100 kg)	60%	Typhimurium
C	Finishing Farm	2500	2 (1000/1500)	20	By pen	70–85 days of life	23	129 days (100 kg)	50%	Typhimurium
D	Finishing Farm	2960	2 (1480/1480)	18	By barn	70–85 days of life	21	102 days (115–120 kg)	92.5%	Typhimurium

[a] Percentage of positive pigs by a commercial ELISA (Herdcheck Salmonella®, Idexx laboratories) using a 40 OD% cut-off value.