Veterinary Microbiology 199 (2017) 91–99

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Effect of enrofloxacin on *Haemophilus parasuis* infection, disease and immune response

Nubia Macedo[a], Maxim C.J. Cheeran[a], Albert Rovira[a], Andrew Holtcamp[b], Montserrat Torremorell[a,*]

[a] College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA
[b] Bayer Animal Health, Shawnee, KS, USA

ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 25 November 2016
Accepted 22 December 2016

Keywords:
Haemophilus parasuis
Enrofloxacin
Immunity

ABSTRACT

Haemophilus parasuis, the causative agent of Glasser's disease, is a pathogen that colonizes the upper respiratory tract (URT) of pigs, invades the bloodstream and causes polyserositis. Because antimicrobials are highly effective against *H. parasuis*, we hypothesized that they could have a detrimental effect on the establishment of an immune response if given at the time of URT colonization. In this study, we characterized clinical outcomes and antibody and IFN-γ responses to *H. parasuis* in pigs treated with enrofloxacin before or after low dose inoculation with a pathogenic *H. parasuis* strain. Pigs that were only inoculated with the agent (EXP group) and pigs that were treated with enrofloxacin and then inoculated (ABT/EXP group) developed signs of disease starting at 4 days post inoculation (DPI), presented a significant increase in serum IgG and were protected against a subsequent homologous challenge. In contrast, pigs treated with antibiotic after inoculation (EXP/ABT group) neither showed signs of disease nor seroconverted (IgG) after low dose inoculation. EXP/ABT pigs as well as naïve control pigs [enrofloxacin only (ABT) and challenge only (CHA)] were susceptible to challenge. Variable levels of antibodies in bronchioalveolar fluid and IFN-γ in peripheral blood mononuclear cells were observed after *H. parasuis* inoculation, but were not associated with protection. In summary, only pigs treated before low dose *H. parasuis* inoculation seroconverted and were protected against subsequent challenge. Results from this study can help determine timing of antimicrobial use and contribute to our current understanding of judicious antibiotic use.

1. Introduction

Haemophilus parasuis is a Gram-negative bacterium that causes Glasser's disease in pigs. The disease is characterized by fibrinopurulent polyserositis, arthritis and meningitis leading to high mortality and morbidity, which results in significant economic losses to pig producers (Aragon et al., 2012). Pathogenic and non-pathogenic *H. parasuis* strains can be isolated from the upper respiratory tract (URT) of healthy pigs (Oliveira et al., 2003; Macedo et al., 2014). Stress conditions such as weaning and transport have been suggested as risk factors for *H. parasuis* systemic dissemination (Aragon et al., 2012). However, the mechanisms involved in the systemic invasion of *H. parasuis* are largely unknown.

Vaccines against *H. parasuis* are available and can be used to prevent infection (Oliveira and Pijoan, 2004). However, control of Glasser's disease through vaccination is difficult, especially because there are many strains and subtypes, and cross-protection between them is limited (Brockmeier et al., 2013). In contrast, field studies have demonstrated that inoculating piglets at a young age with a low dose of a live pathogenic *H. parasuis* strain reduced nursery mortality due to Glasser's disease (Oliveira et al., 2001a, 2004). The mechanism behind such protection was not determined, but it was hypothesized that such exposure in the presence of maternal immunity results in *H. parasuis* colonization, which may elicit a protective immune response without causing disease (Pijoan et al., 1997).

Antimicrobials have been widely used in the swine industry to control bacterial respiratory diseases (Cromwell, 2002). About half of farms in the US with nursery-age pigs use injectable antimicrobials to treat respiratory diseases and feed-grade

* Corresponding author.
E-mail addresses: maced004@umn.edu (N. Macedo), cheeran@umn.edu (M.C.J. Cheeran), rove0010@umn.edu (A. Rovira), andrew.holtcamp@bayer.com (A. Holtcamp), torr0033@umn.edu (M. Torremorell).

http://dx.doi.org/10.1016/j.vetmic.2016.12.032
0378-1135/

antimicrobials are commonly administered to all pigs in the entire room (NAHMS, 2006). Antimicrobials exert a direct deleterious effect on bacterial infections by decreasing the bacterial load, permitting the host to activate immune defenses, and eliminating the pathogen without excessive inflammation (Cromwell, 2002). Specifically, for *H. parasuis,* specific antimicrobials have been shown to be extremely useful in the control and treatment of Glasser's disease (Aragon et al., 2012). On the other hand, there are few reports indicating that antimicrobials can have unintended consequences by preventing the development of immunity. Use of antimicrobials in the early stages of disease prevented the development of protective immunity against reinfection in mice with *Listeria sp., Chlamydia trachomatis* and *Salmonella typhimurium,* and in pigs with *Actinobacillus pleuropneumoniae* (North et al., 1981; Su et al., 1999; Sjolund et al., 2009; Griffin et al., 2009). In contrast, another study demonstrated that a protective antibody response against *S. typhimurium* in mice was primed even after early antibiotic treatment with enrofloxacin (Johanns et al., 2011). Antimicrobial treatment can also affect *H. parasuis* colonization (Vilalta et al., 2012; Macedo et al., 2014). However, the effect of antimicrobials on the development of an effective immune response against *H. parasuis* requires further investigation. Our hypothesis is that antimicrobials will affect *H. parasuis* infection and subsequent development of a protective immune response against *H. parasuis*.

To better study the relationship between *H. parasuis* infection, immunity, protection, and use of antibiotics, we used an animal model where conventional pigs were inoculated with a low dose of a pathogenic *H. parasuis* strain at weaning and were treated with enrofloxacin, followed by homologous challenge with a high dose of *H. parasuis*. This model attempts to mimic field conditions of *H. parasuis* colonization, infection, and antibiotics use.

2. Materials and methods

2.1. Experimental design

Sixty 3-week-old conventional pigs from seven different litters were selected from a herd free of *Mycoplasma hyopneumoniae,* porcine reproductive and respiratory syndrome (PRRS) virus and influenza virus, and without history of Glasser's disease. Pigs were individually identified, weighed and assigned to 6 groups of 10 pigs each, randomizing for sex, weight and litter of origin. Each group was kept in a separate isolation room and pigs were cared for according to University of Minnesota approved Institutional Animal Care and Use Committee (IACUC) protocols.

A summary of the experimental procedures and timeline for each group is shown in Table 1. Three groups of pigs (EXP, EXP/ABT, ABT/EXP) were inoculated with a low dose of pathogenic *H. parasuis* (exposure) on day 0. Group ABT/EXP was also treated with enrofloxacin before inoculation, on day -3 of the study. Group EXP/ABT was treated with enrofloxacin 3 days post inoculation (DPI). Group ABT was treated with enrofloxacin 3 DPI but was not inoculated with low dose of pathogenic *H. parasuis,* and served as a control for the effect of the antibiotic treatment alone. Pigs in the group CHA were only inoculated with a high dose of *H. parasuis* on day 21 (challenge) and served as positive controls. Groups EXP, EXP/ABT, ABT/EXP and ABT were also challenged at 21 DPI. Pigs in the negative control group (NEG) were untreated, non-exposed, and non-challenged. At the termination of the study, all pigs were euthanized at either 25 or 35 DPI.

2.2. Haemophilus parasuis inoculation

Inoculation was done according to Macedo et al. (2016). Briefly, bacterial growth was diluted in phosphate buffered saline (PBS) to produce an inoculum containing 10^6 (exposure at day 0) or 10^8 (challenge at day 21) colony forming units (CFU)/ml of *H. parasuis* Nagasaki reference strain and was administered to pigs in a 1 mL volume, intranasally.

2.3. Antimicrobial treatment

Pigs treated with antimicrobial received a single dose of injectable enrofloxacin (7.5 mg/kg of body weight, Baytril, Bayer Animal Health, Shawnee, Kansas, USA) subcutaneously, either 3 days prior to (ABT/EXP) or post (EXP/ABT) low dose inoculation (Table 1). Pigs not treated with antimicrobial received 1 mL of saline solution. Pigs that had clinical signs of Glasser's disease after low dose inoculation were treated therapeutically with enrofloxacin following manufacturer specifications as described above.

2.4. Sample collection

In order to determine colonization and infection status, nasal swabs for bacterial isolation were collected at -3, 2, 7 and 18 DPI from all pigs in all treatment groups and also at 3 and 5 DPI from pigs in groups EXP, EXP/ABT, and ABT/EXP. Laryngeal swabs were collected from groups EXP, EXP/ABT, and ABT/EXP at 2, 3, 5, 7 and 18 DPI. Swabs from the nasal cavity, trachea, brain, pleura, pericardium, peritoneum, and joints were collected at necropsy for *H. parasuis* isolation. *H. parasuis* isolation was also attempted from lung and liver tissues. Blood samples were collected at 2 and 23 DPI from inoculated pigs to monitor for bacteremia through bacterial isolation and PCR (Oliveira et al., 2001b).

To measure immune responses, serum samples were collected from all pigs at -3, 17, 25, and 35 DPI for serological testing. Whole blood samples were also collected in EDTA (BD Vacutainer™ Glass

Table 1
Experimental design showing *Haemophilus parasuis* inoculation details, enrofloxacin treatment and necropsy time points.

Groups	Number of pigs[a]	Days of study				
		−3	0	3	21	25 or 35
EXP	10		Exposure[c]		Challenge[d]	Necropsy
ABT/EXP	10	Enrofloxacin[b]	Exposure		Challenge	Necropsy
EXP/ABT	10		Exposure	Enrofloxacin	Challenge	Necropsy
ABT	10			Enrofloxacin	Challenge	Necropsy
CHA	10				Challenge	Necropsy
NEG	10					Necropsy

[a] 3-week-old, high-health status pigs.
[b] One dose of 7.5 mg/kg injectable enrofloxacin (Baytril® 100).
[c] Inoculation with *H. parasuis* Nagasaki strain at a low dose of 10^6 CFU/ml.
[d] Inoculation with *H. parasuis* Nagasaki strain at a high dose of 10^8 CFU/ml.