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Effect of marbofloxacin on *Haemophilus parasuis* nasal carriage

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ABSTRACT

Haemophilus parasuis is a colonizer of the upper respiratory tract and the causative agent of Glässer's disease in swine. This study focused on the nasal carriage of *H. parasuis* after treatment with marbofloxacin. Three marbofloxacin treatments (three doses of 2 mg/kg body weight [bw] every 24 h, two doses of 4 mg/kg bw every 48 h and 8 mg/kg bw in one single shot) were used and all of them reduce significantly ($p < 0.05$) the nasal carriage of *H. parasuis* as compared to control animals. Moreover, *H. parasuis* was not detected in the nasal cavities of piglets after administering the highest dose. The effect of a dose of 8 mg marbofloxacin/kg bw in one shot was further studied in a farm with clinical cases of Glässer's disease using a longitudinal study. Statistically significant reduction of nasal carriage of *H. parasuis* was detected during the first week after treatment in comparison with the control group. However, a clear relationship between the minimum inhibitory concentration (MIC) of the different strains, their putative virulence or the treatment group (antibiotic or control) from which they were isolated was not detected. Finally, the effect induced by the antibiotic treatment on the bacterial strains seemed to be transitory, since diverse *H. parasuis* strains (with high and low marbofloxacin MICs) were observed 7 days after finishing the treatment.

1. Introduction

Haemophilus parasuis is a Gram-negative bacterium, member of the family Pasteurellaceae, which produces fibrinous polyserositis, polyarthritis and meningitis (Glässer's disease) in pigs (Rapp-Gabrielson et al., 2006). This bacterium is found in the upper respiratory tract of healthy pigs, which are colonized very early after birth by different genotypes of variable virulence (Harris et al., 1969; Cerdà-Cuéllar et al., 2010). Glässer's disease has an important economic impact in affected herds due to the losses caused by the mortality and/or the cost of the antimicrobial treatments necessary to control the disease.

Marbofloxacin is a second-generation fluoroquinolone only used in veterinary medicine. It possesses a broad spectrum activity against *Mycoplasma*, most Gram negative and some Gram-positive bacteria (Appelbaum and Hunter, 2000). This spectrum of activity includes most of the swine respiratory pathogens, including *H. parasuis* (Vallé et al., 2006). Fluoroquinolones conform to concentration dependency against Gram-negative bacteria and achieve values for specific pharmacokinetics and pharmacodyamics parameters are recommended to prevent bacterial growth during treatment and resistance selection (Dudley, 1991; Thomas et al., 2001).

To our knowledge, there is no information available on how antibiotic treatment affects the nasal carriage of *H. parasuis*. Taking into account that fluoroquinolones are usually used to treat respiratory diseases in pigs, the goals

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of the present work are to study the nasal carriage of *H. parasuis* in pigs treated with different posology regimes of marbofloxacin and how these treatments may affect the presence of different strains of this microorganism before and after the treatment under field conditions.

2. Materials and methods

Two different studies were carried out to meet these goals. They are identified as A and B. Study A evaluated the effect of three different marbofloxacin posology regimes (three doses of 2 mg/kg body weight [bw] every 24 h, two doses of 4 mg/kg bw every 48 h and 8 mg/kg bw in one single shot) in nasal carriage of piglets by *H. parasuis*. The effect of the antimicrobial treatment was determined 24 h after the last marbofloxacin administration. In study B, the nasal carriage of *H. parasuis* in piglets treated with marbofloxacin at 8 mg/kg bw in one single shot was evaluated at different time points.

2.1. Study A: effect of 3 different marbofloxacin posology regimes on H. parasuis carriage

The study was carried out in a commercial farm without clinical cases of Glässer disease. Forty 2-month-old pigs weighting 17.4–27.1 kg were used in this study. Animals were clinically healthy when the study began. Pigs received non-medicated commercial feed ad libitum and had free access to drinking water. Animals were housed in a conventional farm under field conditions in pens containing 13 piglets by pen. The space available for the animals was 0.75 m^2/pig. This density was considered adequate under commercial conditions. The building was equipped with manual mechanisms to control ventilation. Animals were ear tagged with unique numbers and were randomly divided into four groups (control, P1, P2 and P3). Group P1 received Marbocyl 2% (Vetoquinol Laboratory, Lure, France) applied intramuscularly at a dose of 2 mg marbofloxacin/kg bw every 24 h for three consecutive days. Group P2 received Marbocyl 2% intramuscularly at a dose of 4 mg marbofloxacin/kg bw twice with a 48 h interval between treatments. Group P3 received Marbocyl 10% (Vetoquinol Laboratory, Lure, France) at a dose of 8 mg marbofloxacin/kg bw in a single shot intramuscularly. In this later group, Marbocyl 10% was used to reduce the volume injected and the possible adverse reactions such as pain and edema at the injection site. These doses were selected taking into account the summary of product characteristics of Marbocyl® and the most frequently used extra-label posology regimens in use under field conditions (JM Caballero, Laboratorios Vétoquinol, Spain, Personal communication). Animals of the control group were sham injected intramuscularly with the same volume of physiological saline. All the animals housed in the same pen received the same treatment (control, P1, P2 or P3) but only 10 out of 13 were sampled to carry out microbiological determinations. This experimental design was chosen to mimic as much as possible the normal situation under field conditions.

Nasal swabs were taken 24 h after the last antibiotic administration, and transported under refrigeration to the laboratory.

No concurrent medications were administered to the animals during the course of the study.

2.2. Study B: nasal colonization of H. parasuis in piglets treated with marbofloxacin at 8 mg/kg bw in one single shot

The study was carried out in a farm with clinical cases of Glässer's disease, with 1500 sows in a farrow-to-finish production system. A total of 300 4-week-old crossbred pigs were used in this study (150 animals per group). Animals were clinically healthy when the study began. Animals were housed in pens containing 25 animals per pen. The space available per animal was 0.36 m^2/animal. This density was considered adequate under commercial conditions. The building was equipped with automatic mechanism to control ventilation and temperature. Pigs were fed and had water available ad libitum. The feed was distributed in hoppers (one per pen) and the water was supplied through an automated system. All the pigs included in the study received non-medicated feed, which was normally applied under commercial conditions in this farm. Feed was stored at room temperature. Animals were randomly divided in two groups, which were placed in independent rooms with independent ventilation. Treatment group received Marbocyl® 10% (Vétoquinol Laboratory, Lure, France) applied intramuscularly at a dose of 8 mg/kg bw in one shot. Animals of the control group were sham injected intramuscularly with the same volume of physiological saline. Treatments were applied at the beginning of the nursery period. Piglets included in the trial were clinically monitored and mortality was also recorded. A subpopulation of 20 piglets of each group were randomly selected and tagged. Nasal swabs from those selected piglets were taken on days 0, 1, 7, 14 and 28 and transported under refrigeration to the laboratory.

Piglets were observed daily for general health conditions. No concurrent medications were administered to the animals during the course of the study.

2.3. H. parasuis isolation and identification

Collected swabs were plated on chocolate agar (bioMérieux, Madrid, Spain). After 2–3 days at 37 °C with 5% CO_2, all *H. parasuis*-like colonies were selected and subcultured for identification and further analysis. The swabs were also processed for DNA extraction with the Nucleospin blood kit (Macherey-Nagel) following manufacturer instructions and the extracted DNA was used in a species-specific PCR to identify *H. parasuis* (Oliveira et al., 2001).

2.4. Characterization of H. parasuis isolates

Two different PCR were used for characterization of *H. parasuis* isolates: enterobacterial repetitive intergenic consensus (ERIC)-PCR for determination of the different strains in the animals (Olvera et al., 2006) and the virulence-associated trimeric autotransporter (*vtaA*) PCR for determination of the putative virulence of the strains (Olvera et al., 2011).