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Epidemiology and susceptibility of pathogenic bacteria responsible for upper respiratory tract infections in pet rabbits

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Abstract

For 8 months, 121 pet rabbits of more than 2 months old were included in an epidemiological study aimed at determining the nature, prevalence and bacteriological susceptibility of pathogenic bacteria responsible for upper respiratory tract disease (''snuffles''). All rabbits presented with nasal discharge and sneezing at inclusion and had not received any antibiotics in the 30 days prior to the study. Nasal samples were taken from all the rabbits before they received any treatment. Isolation of bacterial strains, susceptibility testing by disk diffusion for marbofloxacin, enrofloxacin, danofloxacin, gentamicin, oxytetracycline, doxycycline, cefalexin, trimethoprim-sulfamethoxazole, and marbofloxacin MIC determination for each pathogenic bacterium were also performed. The main bacterial strains isolated were *Pasteurella multocida* (54.8%), *Bordetella bronchiseptica* (52.2%), *Pseudomonas* spp. (27.9%) and *Staphylococcus* spp. (17.4%). Snuffles was mainly due to a polybacterial infection, and the most frequently found combination was *P. multocida* and *B. bronchiseptica* (28.9% of rabbits). Marbofloxacin was shown to be the most effective agent against all bacterial strains (between 87.8% and 100% susceptibility according to strain) except *B. bronchiseptica*, for which gentamicin was slightly more effective (96% versus 88.9%). Compared to other fluoroquinolones tested, marbofloxacin exhibited the highest level of activity. Marbofloxacin MIC_{90} was equivalent to 1.320, 0.079, 1.741 and 0.490 μg/ml for *B. bronchiseptica*, *P. multocida*, *Pseudomonas* spp. and *Staphylococcus* spp. strains, respectively. In this study, marbofloxacin was shown to be a potentially good treatment option for upper respiratory tract disease in pet rabbits.

Keywords: *Pasteurella multocida*; *Bordetella bronchiseptica*; Snuffles; Pet rabbits; Fluoroquinolones; Marbofloxacin

1. Introduction

Infectious upper respiratory disease, more generally called ''snuffles'', is considered the most common disease observed in pet rabbits (Langan et al., 2000). Common clinical signs include nasal discharge, sneezing and conjunctivitis (Langan et al., 2000; Percy

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and Barthold, 2001; Boucher and Nouaille, 2002). The agent most commonly implicated with these symptoms is *Pasteurella multocida* (Deeb et al., 1990; Broome and Brooks, 1991; Langan et al., 2000). However, other pathogens are cited, such as *Staphylococcus* spp. and *Bordetella bronchiseptica*, although apparently this is by no means an exhaustive list (Langan et al., 2000). Nevertheless, to our knowledge, there are no studies available on the antibiotic susceptibility of bacteria implicated in snuffles in pet rabbits.

The purpose of this study was to identify and to evaluate the prevalence of the pathogenic bacteria responsible for upper respiratory tract infections in pet rabbits of more than 2 months old. The susceptibility of the bacterial strains isolated to eight antibiotics was analysed as was the marbofloxacin MIC distribution.

2. Materials and methods

2.1. Selection of animals

One hundred and twenty one pet rabbits were included in the study by 10 veterinarian investigators in France and the UK between February and September 2004. The pet rabbits selected had to be over 2 months of age and had to display at least the symptoms of nasal discharge and sneezing. They could not have been treated with antibiotics in the 30 days preceding the trial and could not have received a prior vaccine against respiratory tract pathogens.

2.2. Collection of bacterial strains

A nasal swab was taken from both nares of each pet rabbit. Bacterial strains were collected with mini-tip swabs using Amies charcoal transport medium. Specimens were sent within 24 h to two reference laboratories in France and the UK for French and English cases, respectively.

Each sample was plated on selective and conventional media (Biomérieux, Marcy l'Etoile, France) to isolate strains. Petri dishes have been incubated for about 24 h in a culture-appropriate atmosphere and temperature: *Enterobacteriaceae* and *Pseudomonas* spp. on Tryptone Soy Agar, Columbia Blood Agar or MacConkey agar at 35 ± 2 °C; *Pasteurella* spp. on Columbia Blood Agar at 35 ± 2 °C with 6% CO_2; *Staphylococcus* spp. on Tryptone Soy Agar and *Bordetella* spp. on Columbia Blood Agar, both at 35 ± 2 °C; *Streptococcus* spp. and other Gram-positive bacteria on Columbia Blood Agar or TKT Agar (Merck, Darmstadt, Germany) at 35 ± 2 °C. After this isolation stage, the strains were identified by colony morphology, Gram staining characteristics and oxidase (Gram negative bacilli) or catalase (Gram positive cocci) tests. Strains were purified on specific agar media as mentioned before and identified by biochemical API® systems (Biomérieux, Marcy l'Etoile, France): Api 20E for *Enterobacteriaceae*, Api 20E or Api 20NE for *Pasteurella* spp., Api 20NE for *Bordetella* spp. and *Pseudomonas* spp. and Api 20Strep for *Streptococcus* spp. *Staphylococcus aureus* was identified by colony morphology, haemolytic patterns on Columbia Blood Agar, Gram staining characteristics, catalase, positive result at the agglutination system called Slidex Staph Kit® and ID 32 Staph biochemical API® identification system (Biomérieux, Marcy l'Etoile, France). After identification, the bacterial strains were stored on cryobeads (AES, Combourg, France) at about −80 °C.

Aerobic bacteria (see the previously described methodology) and *Mycoplasma* spp. strains (using two methods) were searched on lungs of rabbits that were already dead at inclusion. First, a *Mycoplasma* Agar Base supplemented with *Mycoplasma* supplement (Oxoid, Basingstoke, England) was streaked with a defrosted sample of lung tissue and incubated 48–72 h at 36 ± 2 °C with 6% CO_2. The second method consisted of crushing a small piece of lung tissue in PBS buffer (Gibco, Carlsbad, USA) and to incubate it in sterile *Mycoplasma* Broth to which had been added *Mycoplasma* Supplement (Oxoid, Basingstoke, England) for about 7 days at 36 ± 2 °C with 6% CO_2. *Mycoplasma* spp. strains were isolated on *Mycoplasma* Agar Base to which had been added *Mycoplasma* Supplement (Oxoid, Basingstoke, England) and incubated 48–72 h at 36 ± 2 °C with 6% CO_2 (Boucher et al., 1999).

2.3. Susceptibility testing

Each pathogenic bacterial strain (*Pasteurella* spp., *Bordetella* spp., *Pseudomonas* spp. and *Staphylococcus* spp.) was sent to Vétoquinol for susceptibility testing.