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Experimental study of pathogenicity of *Pasteurella multocida* serogroup F in rabbits

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Abstract

The role of *Pasteurella multocida* serogroup F in inducing disease in rabbits was investigated in this study. Three groups of 12 *Pasteurella*-free rabbits each were intranasally (i.n.), subcutaneously (s.c.), and perorally (p.o.) challenged, respectively. Six rabbits of each group were immunosuppressed using dexamethasone.

Eight rabbits (four of them immunosuppressed) inoculated i.n. showed symptoms of respiratory distress resulting in respiratory failure and died or were euthanized in the terminal stage of the disease 3–6 days post-infection (p.i.). The main pathological findings were fibrinopurulent pleuropneumonia (immunocompetent rabbits) or diffuse haemorrhagic pneumonia (immunosuppressed rabbits). Septicemic syndrome ending with shock occurred in 11 rabbits (6 of them immunosuppressed) inoculated s.c., which died or were euthanized in the terminal stage of the disease 2–3 days p.i. The most significant pathological findings were extensive cutaneous and subcutaneous lesions. All of the p.o. inoculated rabbits survived the challenge showing no clinical signs of the disease and no macroscopic lesions.

The observations in this study indicate that in addition to serogroups A and D of *P. multocida*, serogroup F also can be highly pathogenic for rabbits and therefore might be a cause of considerable economic loss in commercial rabbit production.

Keywords: *Pasteurella multocida*; Serogroup F; Rabbit; Pasteurellosis

1. Introduction

Pasteurellosis caused by *Pasteurella multocida* is one of the most significant bacterial diseases of rabbits and causes considerable economic losses in large production units throughout the world (Takashima et al., 2001). The disease is characterised by various clinical symptoms, including respiratory distress, genital infections, abscesses, otitis, and septicaemia, but infection by *P. multocida* can also appear without manifesting any clinical signs (DeLong and Manning, 1994). To date, only

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serogroup A and, to a lesser extent, serogroup D strains of *P. multocida* have been considered as causative agents of rabbit pasteurellosis (Kawamoto et al., 1990; Dabo et al., 1999).

P. multocida serogroup F was first described in turkeys in the USA (Rimler and Rhoades, 1987). Isolates of this serogroup originated mainly from birds in North America (Rhoades and Rimler, 1987; Wilson et al., 1995) but they also have been found in birds in other parts of the world (Jonas et al., 2001). Serogroup F has been predominantly described as a causative agent of fowl cholera (Rimler, 1987; Jonas et al., 2001) and for a long time it was quite uncommon in mammalian hosts. Nevertheless, findings of this serogroup in some mammalian species (i.e., pigs, sheep and cattle) showing various clinical symptoms have been newly reported by several authors in different parts of the world (Moreno et al., 2003; Davies et al., 2003; Catry et al., 2005). Moreover, we previously reported on a relatively high incidence of serogroup F among rabbit nests in the Czech Republic (Jaglic et al., 2004) and among a total of eight serogroup F rabbit isolates we found seven distinct pulsed-field gel electrophoresis types (Jaglic et al., 2006). This could indicate that the occurrence of serogroup F in rabbits is not the consequence of a coincidental spreading of one clone to a rabbit host. Because of incomplete data concerning the health and/or epizootiological status of the affected rabbit nests, the aim of this study was to evaluate whether serogroup F can be a new potential agent of the rabbit pasteurellosis or whether it is only a commensal bacterium within a rabbit host.

2. Materials and methods

2.1. Bacterial strain and culture media

P. multocida serogroup F rabbit isolate J-4103 (CAPM 6431) was selected for the experimental infection of rabbits. It was of ribotype 1, the predominant ribotype of the serogroup F rabbit isolates (Jaglic et al., 2004). Prior to the infection, the isolate was passaged three times in chicken embryos. The isolate was routinely grown at 37 °C on blood agar (Blood Agar Base No. 2, HiMedia, India) containing 5% sheep blood.

2.2. Experimental infection of rabbits

2.2.1. Rabbits and housing

Animal handling complied with the legal directives of the Czech Republic and with the institution's policy. Specific pathogen-free New Zealand White rabbits of both sexes and weighing 1.8–2 kg were obtained from Charles River Laboratories, Germany GmbH. The rabbits were individually housed in stainless steel cages placed in isolated rooms with a barrier regime and independent ventilation. The rabbits were fed pellets (a complete food mixture for rabbits 4–12 weeks old, Bonagro, Czech Republic) *ad libitum* and had free access to water. The rooms were kept at a temperature of 21 °C, relative humidity of 40–60%, and ventilation of approximately 15 air changes per hour. Before infection, nasal, conjunctival and rectal swabs as well as whole blood were collected for bacteriological examination to ensure *Pasteurella*-free status of the rabbits. Sera were tested for the presence of *P. multocida* IgG and IgM antibodies by an ELISA described below.

2.2.2. Experimental design

A total of 57 rabbits were divided into three challenged groups of 12 rabbits each (according to the route of inoculation) and one negative control group of 21 rabbits. Each group was placed in a separate room. Six rabbits from each challenged group and 12 rabbits from the control group were immunosuppressed by intramuscular administration of dexamethasone (Dexamed, Medochemie LTD, Cyprus) at a dose of 2 mg/kg of body weight. The dose had been established in our preliminary experiment. Dexamethasone was administered three times in 24-h intervals, and the last application was 24 h post-infection (p.i.).

2.2.3. Challenge

The rabbits were challenged with 6×10^4 CFU in 1 ml of phosphate saline buffer (PBS) as follows: (i) intranasally (i.n.) by direct application of the bacterial suspension into nasal cavities using an aerosol applicator, (ii) subcutaneously (s.c.) in the caudal region of the left scapula, and (iii) perorally (p.o.) between the buccal wall and molars. To avoid aspiration of the inoculum during the p.o. application, a neutral sterile puree (wheat flour and semolina) was added to the bacterial suspension. The negative