



HAL
open science

Experimental study of pathogenicity of serogroup F in rabbits

Zoran Jaglic, Edita Jeklova, Lenka Leva, Vladimir Kummer, Zdenka Kucerova, Martin Faldyna, Jarmila Maskova, Katerina Nedbalcova, Pavel Alexa

► **To cite this version:**

Zoran Jaglic, Edita Jeklova, Lenka Leva, Vladimir Kummer, Zdenka Kucerova, et al.. Experimental study of pathogenicity of serogroup F in rabbits. *Veterinary Microbiology*, 2007, 126 (1-3), pp.168. 10.1016/j.vetmic.2007.06.008 . hal-00532277

HAL Id: hal-00532277

<https://hal.science/hal-00532277>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

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

Authors: Zoran Jaglic, Edita Jeklova, Lenka Leva, Vladimir Kummer, Zdenka Kucerova, Martin Faldyna, Jarmila Maskova, Katerina Nedbalcova, Pavel Alexa



PII: S0378-1135(07)00301-X
DOI: doi:10.1016/j.vetmic.2007.06.008
Reference: VETMIC 3724

To appear in: *VETMIC*

Received date: 29-3-2007
Revised date: 28-5-2007
Accepted date: 4-6-2007

Please cite this article as: Jaglic, Z., Jeklova, E., Leva, L., Kummer, V., Kucerova, Z., Faldyna, M., Maskova, J., Nedbalcova, K., Alexa, P., Experimental study of pathogenicity of *Pasteurella multocida* serogroup F in rabbits, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.06.008

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 ORIGINAL RESEARCH PAPER

2

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

4

5 *Zoran Jaglic, Edita Jeklova, Lenka Leva, Vladimir Kummer, Zdenka Kucerova, Martin*
6 *Faldyna, Jarmila Maskova, Katerina Nedbalcova, Pavel Alexa*

7

8 Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic

9

10

11

12

13 Corresponding author (correspondence address):

14 Zoran Jaglic

15 Veterinary Research Institute

16 Hudcova 70, 621 00 Brno

17 Czech Republic

18 Tel.: +420 5 3333 1216

19 Fax: +420 5 4121 1229

20 E-mail address: jaglic@vri.cz

1 Abstract

2

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

7 Eight rabbits (four of them immunosuppressed) inoculated i.n. showed symptoms of
8 respiratory distress resulting in respiratory failure and died or were euthanized in the terminal
9 stage of the disease 3–6 days post-infection (p.i.). The main pathological findings were
10 fibrinopurulent pleuropneumonia (immunocompetent rabbits) or diffuse haemorrhagic
11 pneumonia (immunosuppressed rabbits). Septicemic syndrome ending with shock occurred in
12 11 rabbits (six of them immunosuppressed) inoculated s.c., which died or were euthanized in
13 the terminal stage of the disease 2–3 days p.i. The most significant pathological findings were
14 extensive cutaneous and subcutaneous lesions. All of the p.o. inoculated rabbits survived the
15 challenge showing no clinical signs of the disease and no macroscopic lesions.

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

19

20 *Keywords:* *Pasteurella multocida*; serogroup F; rabbit; pasteurellosis

21

22 Introduction

23

24 Pasteurellosis caused by *Pasteurella multocida* is one of the most significant bacterial
25 diseases of rabbits and causes considerable economic losses in large production units

1 throughout the world (Takashima et al., 2001). The disease is characterised by various clinical
2 symptoms, including respiratory distress, genital infections, abscesses, otitis, and septicaemia,
3 but infection by *P. multocida* can also appear without manifesting any clinical signs (DeLong
4 and Manning, 1994). To date, only serogroup A and, to a lesser extent, serogroup D strains of
5 *P. multocida* have been considered as causative agents of rabbit pasteurellosis (Kawamoto et
6 al., 1990; Dabo et al., 1999).

7

8 *P. multocida* serogroup F was first described in turkeys in the USA (Rimler and Rhoades,
9 1987). Isolates of this serogroup originated mainly from birds in North America (Rhoades and
10 Rimler, 1987; Wilson et al., 1995) but they also have been found in birds in other parts of the
11 world (Jonas et al., 2001). Serogroup F has been predominantly described as a causative agent
12 of fowl cholera (Rimler, 1987; Jonas et al., 2001) and for a long time it was quite uncommon
13 in mammalian hosts. Nevertheless, findings of this serogroup in some mammalian species
14 (i.e., pigs, sheep and cattle) showing various clinical symptoms have been newly reported by
15 several authors in different parts of the world (Moreno et al., 2003; Davies et al., 2003; Catry
16 et al., 2005). Moreover, we previously reported on a relatively high incidence of serogroup F
17 among rabbit nests in the Czech Republic (Jaglic et al., 2004) and among a total of eight
18 serogroup F rabbit isolates we found seven distinct pulsed-field gel electrophoresis types
19 (Jaglic et al., 2006). This could indicate that the occurrence of serogroup F in rabbits is not the
20 consequence of a coincidental spreading of one clone to a rabbit host. Because of incomplete
21 data concerning the health and/or epizootiological status of the affected rabbit nests, the aim
22 of this study was to evaluate whether serogroup F can be a new potential agent of the rabbit
23 pasteurellosis or whether it is only a commensal bacterium within a rabbit host.

24

25 **Materials and methods**

1

2 *Bacterial strain and culture media*

3

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

9

10 *Experimental infection of rabbits*

11

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

23

24 **Experimental design.** A total of 57 rabbits were divided into three challenged groups of 12
25 rabbits each (according to the route of inoculation) and one negative control group of 21

1 rabbits. Each group was placed in a separate room. Six rabbits from each challenged group
2 and 12 rabbits from the control group were immunosuppressed by intramuscular
3 administration of dexamethasone (Dexamed, Medochemie LTD, Cyprus) at a dose of 2 mg/kg
4 of body weight. The dose had been established in our preliminary experiment.
5 Dexamethasone was administered three times in 24-hour intervals, and the last application
6 was 24 hours post infection (p.i).

7
8 **Challenge.** The rabbits were challenged with 6×10^4 CFU in 1 ml of phosphate saline buffer
9 (PBS) as follows: (i) intranasally (i.n.) by direct application of the bacterial suspension into
10 nasal cavities using an aerosol applicator, (ii) subcutaneously (s.c.) in the caudal region of the
11 left scapula, and (iii) perorally (p.o.) between the buccal wall and molars. To avoid aspiration
12 of the inoculum during the p.o. application, a neutral sterile puree (wheat flour and semolina)
13 was added to the bacterial suspension. The negative control rabbits were inoculated with 1 ml
14 of PBS alone as described above (seven rabbits per each route). The clinical status of the
15 challenged and control rabbits, including body weight, rectal temperature, attitude, and chest
16 murmur, was monitored twice daily. Challenged rabbits which were caught up in the terminal
17 stage of the disease and those which survived until 15 days p.i. were euthanized by bleeding
18 from *v. jugularis* under ketamine-xylazine narcosis. At the same time, three
19 immunosuppressed (IS), and two to three immunocompetent (IC) rabbits of the negative
20 control group were also sacrificed.

21
22 **Pathology.** All challenged and control rabbits were examined for the presence of gross and
23 histological lesions. Lungs, heart, liver, spleen and kidneys were histologically monitored in
24 all the rabbits. A part of the changed skin and subcutis of the subcutaneously inoculated
25 rabbits, as well as parts of the stomach, ileum (in the region of Peyer's patches), caecum and

1 colon wall (including mesenteric lymph nodes) of the perorally inoculated rabbits were also
2 histologically examined. The samples were fixed in 10% neutral buffered formalin for 24 h,
3 embedded in paraffin wax, sectioned at 5 μ m, and stained with haematoxylin-eosin and
4 toluidine blue.

5

6 **Bacteriology.** For the bacteriological examination, nasal, conjunctival and rectal swabs as
7 well as whole blood were sampled from the dead rabbits, from rabbits in the terminal stage of
8 the disease prior to their euthanasia, and also 7 and 15 days p.i. from rabbits that survived the
9 challenge (including the negative control rabbits). The blood samples (1 ml) were drawn from
10 the *a. auricularis caudalis* except in the cases of the dead rabbits, from which the blood was
11 obtained by sterile cardiac puncture. Also examined were 1 ml of whole urine (sterile
12 puncture of the bladder) and impression smears of lung, myocardial, liver, spleen and kidney
13 tissues that were obtained from all the challenged and control rabbits at necropsy. Impression
14 smears of cut sections were made on agar plates and streaked for isolation. In the case of the
15 peroral infection, a segment (wall and contents) of the stomach, ileum (the region of Peyer's
16 patches), caecum, and colon, as well as mesenteric lymph nodes, were also included. The
17 samples were cultivated on non-selective and selective (396 U of neomycin and 30 U of
18 bacitracin in 100 ml of the culture medium; Pamycon Biotika, Slovakia) blood agars (Blood
19 Agar Base No. 2, HiMedia, India) containing 5% sheep blood. Suspected bacterial colonies
20 were examined by species- and capsular-specific PCRs (Townsend et al., 1998; Townsend et
21 al., 2001).

22

23 **ELISA.** Sera obtained from the challenged and control rabbits were examined for the
24 presence of *P. multocida* IgG and IgM antibodies. They were collected with the whole blood
25 at the intervals described above. Sera from the dead rabbits were not examined.

1 Bronchoalveolar lavages (BALs) obtained from all the challenged and control rabbits at
2 necropsy were examined for the presence of *P. multocida* IgA antibodies. The lungs were
3 washed with 15 ml of PBS and BALs (supernatants) were separated at 8000 x g for 10 min.
4 The positive control serum and BAL were prepared by intramuscular and intranasal
5 immunisation of New Zealand White rabbits with the serogroup F *P. multocida* isolate J-4103
6 inactivated by formalin. Serum and BAL obtained from a *Pasteurella*-free rabbit were used as
7 negative controls.

8

9 The antigen used in the assay was prepared from the *P. multocida* J-4103 isolate as described
10 by Kawamoto et al. (1994). An indirect ELISA was carried out as described earlier (Klaassen
11 et al., 1985). HRP-conjugated goat anti-rabbit IgG-Fc antibodies (Bethyl Laboratories Inc.,
12 USA) diluted at 1:40,000, and HRP-conjugated goat anti-rabbit IgM- μ antibodies (Abcam
13 Ltd., UK) diluted at 1:10,000 were used for determination of the *P. multocida* IgG and IgM
14 antibodies, respectively. HRP-conjugated goat anti-rabbit IgA- λ antibodies (Abcam Ltd., UK)
15 diluted at 1:40,000 were used for determination of the *P. multocida* IgA antibodies. The
16 positive threshold (cut-off) was calculated as three standard deviations above the mean optical
17 density for serum and BAL samples obtained from the *Pasteurella*-free rabbits (all rabbits
18 prior to challenge and those belonging to the negative control group).

19

20 **Haematology.** Total leukocyte counts in the blood of the euthanized rabbits were determined
21 using the Digicell 500 cell counter (Contraves AG, Switzerland). Differential leukocyte
22 counts were calculated from blood smears stained with May-Grünwald and Giemsa-
23 Romanowski.

24

25 **Lymphocyte subsets.** Lymphocyte subsets in the euthanized rabbits were quantified by

1 indirect staining and flow cytometry as described earlier (Jeklova et al., 2007). Briefly, the
2 blood was stained using a whole-blood lysis technique. Organ samples (spleen, as well as
3 mesenteric and tracheobronchial lymph nodes) were collected into RPMI 1640 medium and
4 cell suspensions were prepared by carefully teasing the lymphoid tissue using two forceps. All
5 cell suspensions were filtered through a fine nylon mesh. Commercially available monoclonal
6 antibodies (mAbs) were used for detecting lymphocyte subsets. For quantifying
7 B-lymphocytes, R-PE conjugated mouse anti-human CD79 α (clone HM57, DakoCytomation,
8 Denmark) was used. Cell suspension was fixed and permeabilized with IntraStain kit
9 (DakoCytomation, Denmark) and labelled according to the manufacturer's instructions. T
10 cells and their subsets were detected using the following anti-rabbit mAbs: anti-CD4
11 (RTH1A, IgG1), anti-CD8 (ISC27A, IgG2a), anti-panT2 (RTH21A, IgG1) and anti-CD45
12 (ISC18A, IgG2a) (VMRD Inc., USA). As a secondary immunoreagent, FITC (dilution 1:100)
13 or R-PE (dilution 1:500) labelled goat anti-mouse conjugates of appropriate subisotypes
14 (Southern Biotechnology Assoc., Inc., Birmingham, AL) were used. Propidium iodide was
15 used to stain DNA of dead and damaged cells to exclude these from analysis. Data were
16 acquired using a FACSCalibur™ flow cytometer (Becton Dickinson, Mountain View, CA)
17 and CELLQuest™ software. Gating was based on forward and right angle scatter signals.

18

19 **Lymphocyte activity.** The activity of lymphocytes isolated from the blood, spleen, and
20 mesenteric and tracheobronchial lymph nodes of the euthanized rabbits was determined using
21 the mitogen-driven lymphocyte transformation test. The density of the mononuclear cell
22 suspensions obtained by gradient centrifugation (Histopaque® -1077, Sigma) was adjusted to
23 10^6 per ml of RPMI 1640 medium supplemented with 10% precolostral calf serum and
24 antibiotics. Cells were incubated in the presence of concanavalin A mitogen (Con A, Sigma,
25 St. Louis, USA) at a concentration of 10 μ g/ml. Cells without added mitogen served as

1 control samples. The microplates were incubated at 37° C in an atmosphere of 5% CO₂ for 3
2 days. Twenty hours before harvesting (FilterMate Harvester, Packard Bioscience Instrument
3 Company, Meriden, USA), 50 µl of medium with ³H-thymidine (5 µCi/ml) was added. The
4 incorporation of ³H-thymidine was analysed by a microplate scintillation & luminescence
5 counter (TopCount NXT™, Packard Bioscience Instrument Company, Meriden, USA). The
6 results were expressed in stimulation indices, which were calculated as the ratio of counts per
7 minute in stimulated samples versus non-stimulated controls.

8

9 **Results**

10

11 *Experimental infection of rabbits*

12

13 Before their use in the experiment, all the rabbits were confirmed as *Pasteurella*-free and
14 were negative for IgG and IgM antibodies against *P. multocida*. During the experiment, all
15 the rabbits belonging to the negative control group remained *Pasteurella* free and
16 serologically negative, and no clinical or pathological signs of the disease were observed
17 among them.

18

19 **Intranasal challenge.** Severe respiratory disease, with development of the first distinct
20 clinical signs 2–3 days p.i., occurred in eight rabbits. All of them died or were euthanized in
21 the terminal stage of the disease 3–6 days p.i. (Table 1). The main clinical signs were fever
22 and respiratory distress resulting in respiratory failure (severe dyspnoea was observed in the
23 terminal stage of the disease in two rabbits). Pathological findings from the clinically diseased
24 rabbits are summarised in Table 2 and in figures 1 and 2. In the four remaining rabbits, which
25 survived the challenge, only slight reddening of the conjunctivas and a weak nasal whistle

1 were sporadically detected. Three of them had weak pulmonary hyperemia accompanied by a
2 slightly increased occurrence of alveolar macrophages.

3

4 *P. multocida* was re-isolated from the following sites or organs of the clinically diseased
5 IC/IS rabbits: nose (n = 4/4), conjunctiva (n = 1/0), lungs (n = 4/4), blood (n = 1/3),
6 myocardium (n = 4/4), liver (n = 2/3), spleen (n = 0/2), and kidneys (n = 1/3). In the cases of
7 the rabbits surviving the challenge, the bacterium was isolated from the nose 7 days (all
8 rabbits) and 15 days (only IS rabbits) p.i., and also from the myocardium of one IS rabbit.

9

10 Serological positivity for *P. multocida* was mainly detected in the surviving rabbits. All of
11 them were positive for IgG 15 days p.i., whereas positivity for IgM was detected 7 days (one
12 IC and one IS rabbit) and 15 days (two IC and one IS rabbit) p.i. Positivity for IgM was also
13 confirmed in one IS rabbit prior to its euthanasia 6 days p.i. This challenge group was also the
14 only one where IgA positive BALs were found. Clear IgA positivity was confirmed in all four
15 rabbits that survived the challenge (15 days p.i.), whereas weakly IgA positive BALs were
16 detected in two IS rabbits that died 6 days p.i. Lymphopenia (mainly due to a decrease of
17 CD8+ lymphocytes) and depression of lymphocyte activity in the blood were mainly observed
18 in the clinically diseased rabbits (Tables S1–S6; see the Supplemental Data).

19

20 **Subcutaneous challenge.** Eleven rabbits, showing the first clinical signs 1 day p.i., became
21 seriously ill and died or were euthanized in the terminal stage of the disease 2–3 days p.i.
22 (Table 1). All the diseased rabbits had an acute septicemic syndrome ending with respiratory
23 symptoms and shock. One of the most significant signs was dark blue to violet or blackish
24 skin rapidly spreading from the place of inoculation and affecting extensive areas of the
25 lateral and ventral abdomen and thorax (including the left thoracic limb). Petechial and

1 ecchymotic haemorrhages, more numerous in the IS rabbits, were mainly located on the ear
2 skin, parietal and visceral pleura, and pericardium. The main pathological findings observed
3 in the clinically diseased rabbits are shown in Table 3 and Figure 3. The sole IC rabbit that
4 survived the challenge showed no signs of the disease. This rabbit was also negative for *P.*
5 *multocida* and the only one in this challenge group in which serological positivity was
6 observed (for IgM 7 and 15 days p.i. and for IgG 15 days p.i.).

7

8 *P. multocida* was re-isolated from the following sites or organs of the clinically diseased
9 IC/IS rabbits: nose (n = 4/2), urine (n = 0/1), blood, lungs, myocardium, liver, spleen, and
10 kidneys (all rabbits). Among these rabbits, lymphopenia and decreased lymphocyte activity in
11 the blood and monitored lymphoid organs were also observed (Tables S1–S6; see the
12 Supplemental Data).

13

14 **Peroral challenge.** All the perorally inoculated rabbits survived the challenge showing no
15 clinical signs of the disease and no macroscopic lesions. Only enlarged Peyer's patches were
16 found in five of them, the alteration of which was histologically confirmed as catarrhal
17 nonpurulent ileitis. However, such changes may not have been solely induced by *P.*
18 *multocida*. *P. multocida* was not recovered from any of the p.o. challenged rabbits but
19 positivity for IgM antibodies 7 and 15 days p.i. was found in two of them (one IC and one IS
20 rabbit). Even though all the perorally inoculated rabbits survived, decreased lymphocytes
21 activity was found in the blood and monitored lymphoid organs in both the IC and IS animals.
22 CD4+8+ double positive cells significantly increased in the blood, spleen and
23 tracheobronchial lymph nodes of the IC rabbits (Tables S1–S6; see the Supplemental Data).

24

25 **Discussion**

1
2 The different routes of inoculation tested in this study resulted in different prevalences of the
3 disease among the challenged rabbits. By the i.n. inoculation we aimed to reproduce a natural
4 route of infection that is the most common in mammalian hosts (Adlam and Rutter, 1989).
5 Subcutaneous inoculation bypasses the external immunological barriers and therefore it is not
6 surprising that the highest morbidity occurred among the s.c. inoculated rabbits. However,
7 after administering a serogroup A *P. multocida* strain at different challenge doses, Lu et al.
8 (1982a) reported a higher mortality among i.n. inoculated rabbits (up to 75%) than in s.c.
9 inoculated rabbits (17–50%). Finally, as serogroup F has been mainly described as a causative
10 agent of fowl cholera and where transmission by aerosol has been reported to be less
11 important (Rhoades and Rimler, 1989), we also tested the peroral route of infection that has
12 been successfully used in an avian host (Pehlivanoglu et al., 1999). This route, however,
13 failed to induce the disease in any of the challenged rabbits, indicating that peroral intake does
14 not play an important role in transmitting the tested isolate.

15
16 Pasteurellosis of rabbits may break out under the influence of various stressors that suppress
17 the animals' immunity (Webster, 1924; DeLong and Manning, 1994). We therefore tried to
18 simulate such immunosuppression by administering dexamethasone, which in our preliminary
19 experiment resulted in lymphopenia associated with lymphocyte redistribution to lymph
20 nodes and other lymphatic tissues (including bone marrow) and depression of lymphocyte
21 activity (unpublished data). As a relatively high prevalence of the fatal disease was observed
22 in both the IC and IS rabbits (except for those p.o. challenged), however, it could be assumed
23 that the isolate tested in this study is highly virulent. Conversely, Lu et al. (1982b), using a
24 serogroup A *P. multocida* strain, observed a significantly higher prevalence of pneumonia in
25 hydrocortisone-treated rabbits (80%) than in non-treated rabbits (20%). The conclusion on

1 high virulence of the challenge isolate also can be supported by the finding that most of the
2 i.n. challenged rabbits became seriously ill even though they were inoculated with a relatively
3 low challenge dose. In general, many other authors have used quite higher challenge doses
4 (10^6 – 10^{10} CFU) in i.n. experimental infections of rabbits with *P. multocida* serogroup A
5 strains (DiGiacomo et al., 1987; Glavits and Magyar, 1990; Lu et al., 1991; Al Haddawi et al.,
6 2000). Failure to induce the disease in rabbits by i.n. application of *P. multocida* serogroup A
7 and D strains at a dose of 10^5 CFU has been described (DeLong et al., 1992). Percy et al.
8 (1986) speculated that the upper respiratory tract serves as an effective barrier that protects
9 the animals from the disease and pulmonary lesions.

10

11 In the i.n. challenged rabbits, the most significant pathological changes were observed in the
12 lungs. In contrast to a study in which essentially the same pulmonary lesions were described
13 in both hydrocortisone-treated and non-treated rabbits (Lu et al., 1982b), the pathological
14 findings in lungs of IC and IS rabbits were generally different. In the IC rabbits, and as
15 already observed elsewhere (Al Haddawi et al., 2000), inflammatory events played an
16 important role in development of the pulmonary lesions. The fact that just evoking the
17 inflammatory reaction, which does not have to be induced by the microorganism itself, could
18 lead to pulmonary lesions was previously reported by Warner et al. (1988), who had used a
19 derived endotoxin alone. On the other hand, the diseased rabbits (with one exception) having
20 suppressed inflammatory response had diffusively spread pulmonary haemorrhages without
21 the characteristic inflammatory lesions. In this challenged group, bacteraemia or septicemic
22 disease also occurred, since *P. multocida* was found not only in the lungs but also in the blood
23 and parenchymatous organs. The fact that septicemic pasteurellosis in rabbits could be a fatal
24 sequel especially to rhinitis and pneumonia has been reported (Webster, 1924).

25

1 The most severe pathological changes found in the s.c. challenged rabbits occurred in the
2 cutis and subcutis. To our knowledge, such diffuse and extensive lesions were quite unusual,
3 since mainly localised (sub)cutaneous abscesses have been observed in both naturally infected
4 and subcutaneously injected rabbits (Lu et al., 1982a; DiGiacomo et al., 1983; DeLong and
5 Manning, 1994). Extensive subcutaneous haemorrhages and oedema have been primarily
6 found in ruminants suffering from septicemic pasteurellosis (Carter and De Alwis, 1989;
7 Carrigan et al., 1991). Furthermore, severe cellulitis and dermal lesions caused by *P.*
8 *multocida* have been mainly described in humans (Brue et al., 1992) and birds (Rhoades and
9 Rimler, 1989; Jeffrey et al., 1993). The rapid expansion and extensiveness of the
10 subcutaneous lesions observed in this study indicate that the challenge isolate, which was
11 previously confirmed as dermonecrotxin negative (Jaglic et al., 2005), is highly invasive. In
12 the s.c. challenged rabbits, the lungs were also affected and positive for *P. multocida*. Since
13 the intravenous inoculation of *P. multocida* was reported as a route that could produce acute
14 pneumonic lesions in rabbits (Percy et al., 1986), it could be assumed the lungs may be a
15 target organ without regard to the route of infection.

16

17 All of the diseased i.n. challenged rabbits and 54.5% of those inoculated subcutaneously had
18 *P. multocida* positive nasal swabs. A frequent presence of *P. multocida* in the nose and its
19 general absence in other excretions indicate that nasal discharge could be one of the most
20 important primary sources of the infection. Therefore, airborne spreading or direct contact
21 could be assumed as the main routes of transmission of the disease, as mentioned previously
22 (DeLong and Manning, 1994). *P. multocida* was also recovered from the noses of rabbits that
23 survived the i.n. challenge. While the positivity for *P. multocida* disappeared in the IC rabbits
24 after 7 days p.i., however, those rabbits with suppressed immunity were still positive 15 days
25 p.i. A longer persistence of *P. multocida* in the IS rabbits indicates that immunity-suppressing

1 conditions (e.g. stress) could contribute to the occurrence of bacilli carriers among the
2 animals.

3

4 The serological examination showed that production of the antibodies against *P. multocida*
5 was detectable by ELISA 6–7 days p.i. In general, no significant correlation was observed
6 between the antibody production and immunosuppression. The finding of the *P. multocida*
7 antibodies in those rabbits surviving the i.n. and s.c. challenges shows that the microorganism
8 colonised these animals and interacted with them. On the other hand, an absence of the
9 specific antibodies in most of the p.o. challenged rabbits (accompanied by an absence of *P.*
10 *multocida* in all of the samples collected) could support speculation that the tested isolate is
11 generally unable to colonise the rabbit host by this route.

12

13 Immunological examinations revealed that lymphocyte depression was characteristic for the
14 clinically diseased i.n. and s.c. inoculated rabbits. Transient suppression of immune functions
15 during the acute stage of inflammation prevents excessive damage of self tissues.
16 Uncontrolled production of proinflammatory cytokines may lead to severe
17 immunopathological changes and even be a threat to life. For this reason, it is essential that
18 their production be accurately regulated by a feedback system. The same regulatory system,
19 however, causes the suppression of a mitogen-stimulated lymphocyte transformation.
20 Production of interleukins 10 and 4, together with transforming growth factor-beta, is
21 suspected to be responsible for the anti-inflammatory effects (Oswald et al., 1992). Curiously,
22 however, a decreased activity of lymphocytes was also detected in all p.o. inoculated rabbits
23 even though they survived without observable signs of the disease.

24

25 **Conclusion**

1

2 Although it they cannot be applied for the *P. multocida* serogroup F in general, the
3 observations in this study indicate that this serogroup can be highly pathogenic for rabbits and
4 therefore might be a cause of considerable economic loss in commercial rabbit production.

5 This speculation should especially be taken into account because of the fact that different
6 clones of the serogroup F already have spread among rabbit nests in the Czech Republic.

7

8 **Acknowledgements**

9

10 We would like to thank Eva Kotlarova and Romana Ondriasova for their indispensable
11 technical assistance. We would also like to thank RNDr. Ivan Rychlik, Ph.D. for his critical
12 reading of the manuscript and Dr. Tony Mileman, Ph.D. for English proofreading.

13 This work was supported by grant no. 524/05/2714 from the Grant Agency of the Czech
14 Republic and by project MZE0002716201 of the Ministry of Agriculture (Czech Republic).

15

16 **Supplemental Data**

17

18 Supplemental Data showing comprehensive results of the immunological investigations are
19 available at <http://www.>

20

21 **References**

22

23 Adlam, C., Rutter, J.M., 1989. *Pasteurella* and pasteurellosis. Academic Press, London, pp.
24 1–341.

- 1 Al Haddawi, M.H., Jasni, S., Zamri-Saad, M., Mutalib, A.R., Son, R., Sheikh-Omar, A.R.,
2 2000. Ultrastructural observation of nasal and pulmonary intracellular *Pasteurella*
3 *multocida* A : 3 in rabbits. Vet. Res. Commun. 24, 153–167.
- 4 Brue, C., Chosidow, O., Gueorguieva, E., Girard-Pipau, F., Rahmouni, A., Roujeau, J.C.,
5 Revuz, J., 1992. Cellulites graves à *Pasteurella multocida* : deux observations. Ann.
6 Dermatol. Venereol. 119, 859–860.
- 7 Carrigan, M.J., Dawkins, H.J.S., Cockram, F.A., Hansen, A.T., 1991. *Pasteurella multocida*
8 septicemia in fallow deer (*Dama dama*). Aus. Vet. J. 68, 201–203.
- 9 Carter, G.R., De Alwis, M.C.L., 1989. Haemorrhagic septicaemia. In: Adlam, C., Rutter, J.M.
10 (Eds.), *Pasteurella* and Pasteurellosis, Academic Press, London, pp. 131–160.
- 11 Catry, B., Chiers, K., Schwarz, S., Kehrenberg, C., Decostere, A., de Kruif, A., 2005. Fatal
12 peritonitis caused by *Pasteurella multocida* capsular type F in calves. J. Clin. Microbiol.
13 43, 1480–1483.
- 14 Dabo, S.M., Confer, A.W., Montelongo, M., Lu, Y.S., 1999. Characterization of rabbit
15 *Pasteurella multocida* isolates by use of whole-cell, outer-membrane, and polymerase
16 chain reaction typing. Lab. Anim. Sci. 49, 551–559.
- 17 Davies, R.L., Watson, P.J., Caffrey, B., 2003. Comparative analyses of *Pasteurella multocida*
18 strains associated with the ovine respiratory and vaginal tracts. Vet. Rec. 152, 7–10.
- 19 DeLong, D., Manning, P.J., 1994. Bacterial diseases. In: Manning, P.J., Ringler D.H.,
20 Newcomer, C.E. (Eds.), *The biology of the laboratory rabbits*, 2nd, Academic Press, Inc.,
21 San Diego, pp. 129–170.
- 22 DeLong, D., Manning, P.J., Gunther, R., Swanson, D.L., 1992. Colonization of rabbits by
23 *Pasteurella multocida* - serum IgG responses following intranasal challenge with
24 serologically distinct isolates. Lab. Anim. Sci. 42, 13–18.

- 1 DiGiacomo, R.F., Garlinghouse, L.E., Van Hoosier, G.L., 1983. Natural history of infection
2 with *Pasteurella multocida* in rabbits. J. Am. Vet. Med. Assoc. 183, 1172–1175.
- 3 DiGiacomo, R.F., Jones, C.D.R., Wathes, C.M., 1987. Transmission of *Pasteurella multocida*
4 in rabbits. Lab. Anim. Sci. 37, 621–623.
- 5 Glavits, R., Magyar, T., 1990. The pathology of experimental respiratory infection with
6 *Pasteurella multocida* and *Bordetella bronchiseptica* in rabbits. Acta Vet. Hung. 38, 211–
7 215.
- 8 Jaglic, Z., Kucerova, Z., Nedbalcova, K., Hlozek, P., Bartos, M., 2004. Identification of
9 *Pasteurella multocida* serogroup F isolates in rabbits. J. Vet. Med. B. 51, 467–469.
- 10 Jaglic, Z., Kucerova, Z., Nedbalcova, K., Kulich, P., Alexa, P., 2006. Characterisation of
11 *Pasteurella multocida* isolated from rabbits in the Czech Republic. Vet Med-Czech. 51,
12 278–287.
- 13 Jaglic, Z., Kucerova, Z., Nedbalcova, K., Pavlik, I., Alexa, P., Bartos, M., 2005.
14 Characterisation and comparison of *Pasteurella multocida* isolated from different species
15 in the Czech Republic: capsular PCR typing, ribotyping and dermonecrotxin production.
16 Vet Med-Czech. 50, 345–354.
- 17 Jeffrey, J.S., Shivaprasad, H.L., Duran, L., Cardona, C.J., Charlton, B.R., 1993. Facial
18 cellulitis associated with fowl cholera in commercial turkeys. Avian Dis. 37, 1121–1129.
- 19 Jeklova, E., Leva, L., Faldyna, M., 2007. Lymphoid organ development in rabbits: Major
20 lymphocyte subsets. Dev. Comp. Immunol. 31, 632–644. (*in press*)
- 21 Jonas, M., Morishita, T.Y., Angrick, E.J., Jahja, J., 2001. Characterization of nine *Pasteurella*
22 *multocida* isolates from avian cholera outbreaks in Indonesia. Avian Dis. 45, 34–42.
- 23 Kawamoto, E., Sawada, T., Sato, T., Suzuki, K., Maruyama, T., 1994. Comparison of indirect
24 hemagglutination test, gel-diffusion precipitin test, and enzyme-linked immunosorbent

- 1 assay for detection of serum antibodies to *Pasteurella multocida* in naturally and
2 experimentally infected rabbits. Lab. Anim.-UK. 28, 19–25.
- 3 Kawamoto, E., Sawada, T., Suzuki, K., Maruyama, T., 1990. Serotypes of *Pasteurella*
4 *multocida* isolates from rabbits and their environment in Japan. Nippon Juigaku Zasshi.
5 52, 1277–1279.
- 6 Klaassen, J.M., Bernard, B.L., DiGiacomo, R.F., 1985. Enzyme-linked immunosorbent assay
7 for immunoglobulin G antibody to *Pasteurella multocida* in rabbits. J. Clin. Microbiol. 21,
8 617–621.
- 9 Lu, Y.S., Lai, W.C., Pakes, S.P., Stefanu, C., 1991. The outer membrane of *Pasteurella*
10 *multocida* 3:A protects rabbits against homologous challenge. Infect. Immun. 59, 4517–
11 4523.
- 12 Lu, Y.S., Pakes, S.P., Rehg, J.E., 1982a. Pathogenesis of serotype 3:A *Pasteurella multocida*
13 administered intranasally or subcutaneously in rabbits. Lab. Anim. Sci. 32, 441–441.
- 14 Lu, Y.S., Pakes, S.P., Rehg, J.E., Ringler, D.H., 1982b. Pathogenicity of a serotype 12:A
15 *Pasteurella multocida* in hydrocortisone treated and nontreated rabbits. Lab. Anim. Sci.
16 32, 258–262.
- 17 Moreno, A.M., Baccaro, M.R., Ferreira, A.J.P., de Castro, A.F.P., 2003. Use of single-
18 enzyme amplified fragment length polymorphism for typing *Pasteurella multocida* subsp
19 *multocida* isolates from pigs. J. Clin. Microbiol. 41, 1743–1746.
- 20 Oswald, I.P., Gazzinelli, R.T., Sher, A., James, S.L., 1992. Il-10 synergizes with Il-4 and
21 transforming growth factor-beta to inhibit macrophage cytotoxic activity. J. Immunol.
22 148, 3578–3582.
- 23 Pehlivanoglu, F., Morishita, T.Y., Aye, P.P., Porter, R.E., Angrick, E.J., Harr, B.S.,
24 Nersessian, B., 1999. The effect of route of inoculation on the virulence of raptorial

- 1 *Pasteurella multocida* isolates in Pekin ducks (*Anas platyrhynchos*). Avian Dis. 43, 116–
2 121.
- 3 Percy, D.H., Bhasin, J.L., Rosendal, S., 1986. Experimental pneumonia in rabbits inoculated
4 with strains of *Pasteurella multocida*. Can. J. Vet. Res. 50, 36–41.
- 5 Rhoades, K.R., Rimler, R.B., 1987. Capsular groups of *Pasteurella multocida* isolated from
6 avian hosts. Avian Dis. 31, 895–898.
- 7 Rhoades, K.R., Rimler, R.B., 1989. Fowl cholera. In: Adlam, C., Rutter, J.M. (Eds.),
8 *Pasteurella* and pasteurellosis, Academic Press, London, pp. 95–113.
- 9 Rimler, R.B., 1987. Cross-protection factor(s) of *Pasteurella multocida*: passive
10 immunization of turkeys against fowl cholera caused by different serotypes. Avian Dis.
11 31, 884–887.
- 12 Rimler, R.B., Rhoades, K.R., 1987. Serogroup F, a new capsule serogroup of *Pasteurella*
13 *multocida*. J. Clin. Microbiol. 25, 615–618.
- 14 Takashima, H., Sakai, H., Yanai, T., Masegi, T., 2001. Detection of antibodies against
15 *Pasteurella multocida* using immunohistochemical staining in an outbreak of rabbit
16 pasteurellosis. J. Vet. Med. Sci. 63, 171–174.
- 17 Townsend, K.M., Boyce, J.D., Chung, J.Y., Frost, A.J., Adler, B., 2001. Genetic organization
18 of *Pasteurella multocida* cap loci and development of a multiplex capsular PCR typing
19 system. J. Clin. Microbiol. 39, 924–929.
- 20 Townsend, K.M., Frost, A.J., Lee, C.W., Papadimitriou, J.M., Dawkins, H.J.S., 1998.
21 Development of PCR assays for species- and type-specific identification of *Pasteurella*
22 *multocida* isolates. J. Clin. Microbiol. 36, 1096–1100.
- 23 Warner, A.E., DeCamp, M.M., Molina, R.M., Brain, J.D., 1988. Pulmonary removal of
24 circulating endotoxin results in acute lung injury in sheep. Lab. Invest. 59, 219–230.

- 1 Webster, L.T., 1924. The epidemiology of a rabbit respiratory infection: I. Introduction. J.
2 Exp. Med. 34, 837–841.
- 3 Wilson, M.A., Duncan, R.M., Nordholm, G.E., Berlowski, B.M., 1995. *Pasteurella multocida*
4 isolated from wild birds of North America - a serotype and DNA fingerprint study of
5 isolates from 1978 to 1993. Avian Dis. 39, 587–593.

Accepted Manuscript

1 Table 1. Time-related numbers of euthanized or dead rabbits after challenge with *Pasteurella multocida* J-4103 and numbers of rabbits surviving
 2 the challenge

3

	Intranasal challenge				Subcutaneous challenge			Peroral challenge
	Euthanasia / Exitus			Survived	Euthanasia / Exitus		Survived	Survived
	day 3	day 5	day 6		day 2	day 3		
Immunocompetent rabbits	1 / 0	0 / 0	2 / 1	2	1 / 0	1 / 3	1	6
Immunosuppressed rabbits	1 / 0	0 / 1	1 / 1	2	3 / 3	0 / 0	0	6
Total	2 / 0	0 / 1	3 / 2	4	4 / 3	1 / 3	1	12

1 Table 2. Numbers of animals with occurrence of pathological lesions among the clinically
2 diseased intranasally challenged rabbits (n = 8).

	Lungs ¹⁾	Heart ²⁾	Others ³⁾		
			Liver	Spleen	Kidneys
Immunocompetent rabbits	4	3	2	3	4
Immunosuppressed rabbits	4	2	3	2	3
Total	8	5	5	5	7

3

4 ¹⁾ Severe fibrinopurulent and necrotizing pleuropneumonia in immunocompetent rabbits and diffuse
5 haemorrhagic pneumonia in immunosuppressed rabbits (one immunosuppressed rabbit showed
6 pulmonary changes similar to those observed in immunocompetent rabbits). ²⁾ Dilatation of the right
7 heart ventricle. ³⁾ Congestions, haemorrhages, inflammatory cell infiltrations, and initial dystrophy
8 (more obvious in kidneys).

9

10

11 Table 3. Numbers of animals with occurrence of pathological lesions among the clinically
12 diseased subcutaneously challenged rabbits (n = 11).

	Cutis and subcutis ¹⁾	Lungs ²⁾	Heart ³⁾	Others ⁴⁾		
				Liver	Spleen	Kidneys
Immunocompetent rabbits	5	4	3	4	4	4
Immunosuppressed rabbits	6	5	5	5	5	5
Total	11	9	8	9	9	9

13

14 ¹⁾ Significant and confluent haemorrhages with perivascular cell infiltrations rapidly spreading from
15 the place of inoculation and affecting extensive areas mainly of the thorax and abdomen. ²⁾ Pulmonary
16 hyperemia with moderate infiltration of alveoli with erythrocytes and inflammatory cells. ³⁾ Hyperemia
17 and haemorrhages in myocardium, epicardium and endocardium (also hydropericardium in
18 immunosuppressed rabbits and acute purulent myocarditis in one immunocompetent rabbit). ⁴⁾
19 Congestions, haemorrhages, inflammatory cell infiltrations, and initial dystrophy.

1 **Figure list:**

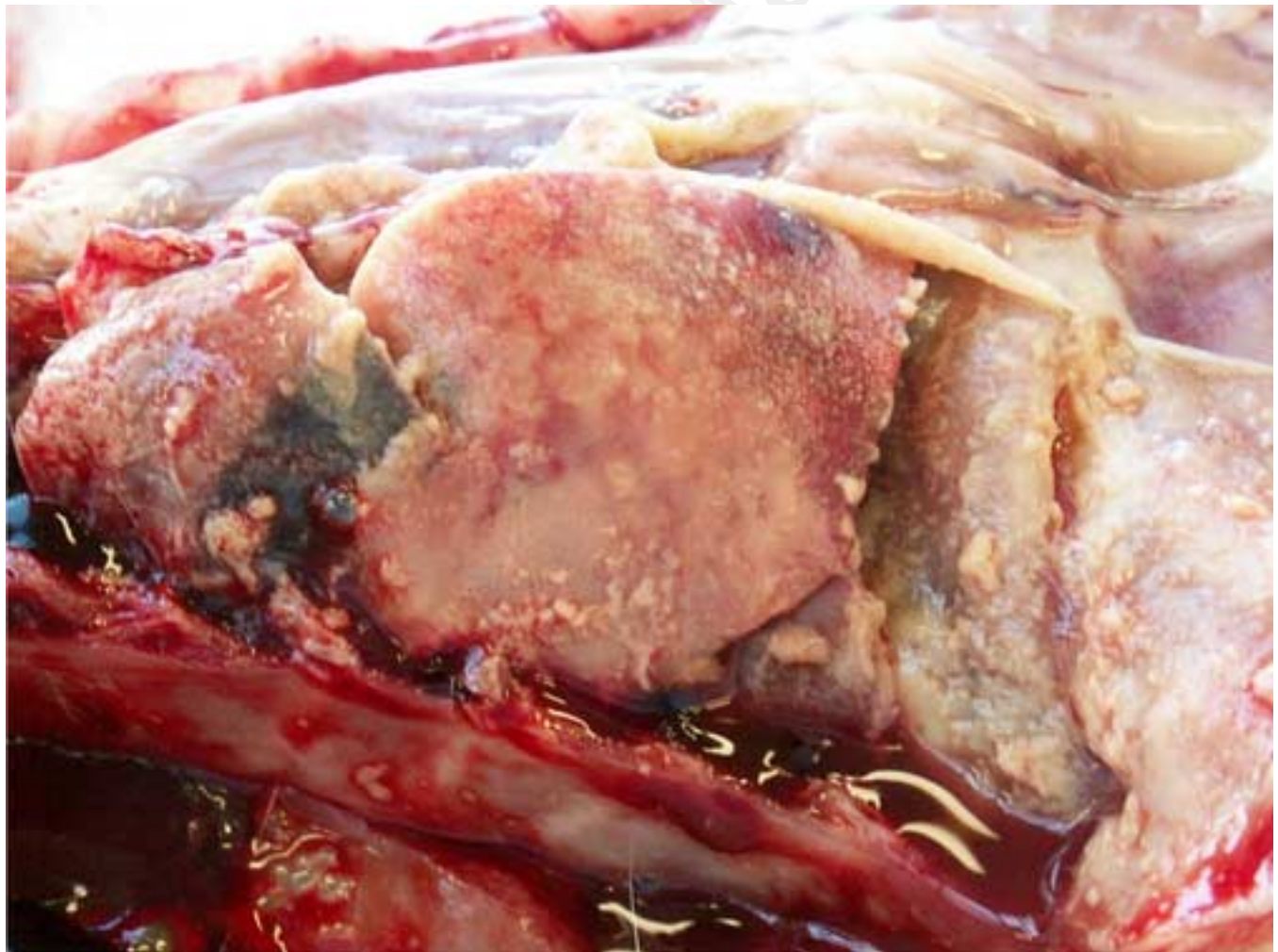
2

3 Fig. 1. Severe fibrinopurulent pleuropneumonia with extensive fibrinous adhesions on parietal
4 pleura and lung surfaces typical for the intranasally challenged IC rabbits.
5 Serohaemorrhagic exudate in thorax also could be observed. Alveoli (and some
6 bronchioli) were partly or completely filled with fibrin and mixed cellular infiltrate
7 (erythrocytes, leukocytes, and desquamated epithelial cells).

8 Fig. 2. Pulmonary hyperemia and haemorrhagic pneumonia occurring in the intranasally
9 challenged IS rabbits. Moderate to very extensive penetration of erythrocytes into the
10 alveolar lumen was the only relevant histological finding.

11 Fig. 3. Very extensive haemorrhages in subcutis of the subcutaneously inoculated IC rabbit
12 (oedematous and “dirty-coloured” subcutis was more characteristic for the IS rabbits).
13 These changes penetrated the abdominal wall and affected the contiguous parietal
14 peritoneum.

Manuscript



Manuscript



Manuscript

