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INFECTIOUS DISEASE

Pyogranulomatous Pleuropneumonia and Mediastinitis in Ferrets (*Mustela putorius furo*) associated with *Pseudomonas luteola* Infection

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Summary

Between 2008 and 2009, three pet ferrets from different sources presented with acute episode of dyspnoea. Cytological examination of pleural exudates revealed severe purulent inflammation with abundant clusters of rod-shaped microorganisms with a clear surrounding halo. Treatment was ineffective and the ferrets died 2–5 days later. Two ferrets were subjected to necropsy examination, which revealed pyothorax, mediastinal lymphadenopathy and multiple white nodules (1–2 mm) in the lungs. Microscopical examination showed multifocal necrotizing-pyogranulomatous pleuropneumonia and lymphadenitis with aggregates of encapsulated microorganisms, some of which were positively stained by periodic acid–Schiff and alcian blue. In-situ hybridization for *Pneumocystis* spp., Ziehl–Neelsen staining and immunohistochemistry for distemper, coronavirus and influenza antigen were negative in all cases. Electron microscopically, the bacteria were 2–3 μm long with a thick electron-lucent capsule. Microbiology from one ferret yielded a pure culture of gram-negative bacteria identified phenotypically as *Pseudomonas luteola*. This speciation was later confirmed by 16S RNA gene amplification.

Keywords: ferret; lymphadenitis; pleuropneumonia; *Pseudomonas luteola*

Introduction

Pseudomonas (*Chryseomonas*) *luteola* is a motile aerobic gram-negative rod with yellow–orange pigmentation of colonies. Its normal habitat is unknown, but it is frequently found in water, soil and other damp environments (Chihab *et al*., 2004). It contains a polysaccharide capsule and multitrichous polar flagella. The capsule has been associated with adsorption of cadmium and cobalt ions (Hawkins *et al*., 1991; Kostman *et al*., 1991; Ozdemir *et al*., 2005).

P. luteola may cause septicaemia, peritonitis and endocarditis in human patients with underlying disease. The organism may also behave as a nosocomial agent and infect critically ill patients who have undergone surgical procedures and/or had indwelling devices inserted. The infection has also been associated with factors such as immunosuppressive therapy, chronic renal failure and malignancy (Chihab *et al*., 2004). Recently, this organism has been described as causing an unusual clinical infection mimicking mediastinal malignant lymphoma in a human patient (Goteri *et al*., 2010). The only report of *P. luteola* infection in the veterinary literature relates to infection of rainbow trout, which developed septicaemia with haemorrhage in the fins and vent and necrotic foci in the liver and kidney (Altinok *et al*., 2007).

In recent years there has been an increase in newly-described or emerging infectious diseases in ferrets, particularly systemic coronavirus infection (Garner

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et al., 2008; Martínez *et al.*, 2008) and influenza (Munster *et al.*, 2009). The present report describes a severe respiratory infection characterized by pyogranulomatous pleuropneumonia, lymphadenitis and mediastinitis associated with *P. luteola* in three ferrets (*Mustela putorius furo*). To our knowledge this is the first report of *P. luteola* infection in warm-blooded animals.

Materials and Methods

Animals

Between 2008 and 2009, three unrelated domestic ferrets (animal numbers 1, 2 and 3) kept as pets by different owners in the metropolitan area of Barcelona, Spain, presented with respiratory disease. All three were males, one was neutered (number 1) and none had contact with other ferrets or animals. Ferrets 1 and 2 were 3 years old and ferret 3 was 2 years old. The animals were not vaccinated against canine distemper virus or rabies. Ferrets 1 and 2 presented with depression, dehydration, anorexia, hyperthermia and acute onset dyspnoea. There was no history of prior medical problems. Complete blood counts and serum biochemical examinations revealed anaemia, severe neutrophilic leucocytosis with toxic neutrophils, hyperglycaemia and hypoalbuminaemia. Ferret 2 also had hyperglobulinaemia. Radiographic findings included a unilateral pleural effusion (right- and left-sided in ferrets 1 and 2, respectively) and a mediastinal mass displacing the trachea dorsally. Ultrasonographically, in ferret 2 there was a large quantity of hypoechoic effusion within the thorax and a heterogeneous ill-defined soft tissue mass was observed in the left mediastinum. Approximately 20 ml of purulent fluid were removed by thoracocentesis. Both ferrets were treated similarly with oxygen supplementation, crystalloid fluids, antibiotics (enrofloxacin, clindamycin and metronidazole), itraconazole, buprenorphine, ranitidine and sucralphate. Both animals died within 2–5 days of presentation.

Ferret 3 had a history of recurrent and vaguely-defined problems consisting mainly of cough. The animal presented with acute, severe dyspnoea and was humanely destroyed without diagnosis or treatment at the request of the owner.

Cytological Examination

Smears of pleural exudates were made from each of the ferrets and these were stained with Diff-Quik.

Gross Examination

Two ferrets were subject to necropsy examination. Ferret 1 was submitted to the Pathology Department at the Veterinary School of Barcelona; ferret 3 was examined by the referring clinician and tissue samples were submitted to a private diagnostic pathology laboratory. Tissue samples from mediastinal lymph nodes, lung, heart, liver, kidney, spleen, pancreas, intestine and brain were collected from ferret 1. Samples of lung, mediastinal lymph node, mediastinal mass, trachea and oesophagus were taken from ferret 3. Samples were fixed in 10% neutral buffered formalin and processed routinely.

Histopathology, Immunohistochemistry and In-situ Hybridization

Formalin-fixed tissues were embedded in paraffin wax, sectioned (4 μm) and stained with haematoxylin and eosin (HE). Gram, Ziehl–Neelsen (ZN), alcian blue and periodic acid–Schiff (PAS) stains were also performed on serial sections from ferrets 1 and 3. Gram, ZN and PAS staining was performed on the pleural exudate of ferret 2.

Immunohistochemistry (IHC) was used to detect ferret systemic coronavirus (FRSCV) antigen (Custom Monoclonals International, Sacramento, CA, USA; dilution 1 in 400), canine distemper virus (CDV) nucleoprotein (Ingenasa, Madrid, Spain; dilution 1 in 1,000) and influenza A (IA) nucleoprotein (CReSA, Barcelona, Spain; dilution 1 in 100) in lung and mediastinal tissues from ferrets 1 and 3, following the protocols previously described (Domingo *et al.*, 1992; Gooskens *et al.*, 2007; Martínez *et al.*, 2008). Negative control procedures included omission of primary antiserum. Tissue sections known to be negative and positive for expression of FRSCV, CDV and IA were included as additional controls.

For in-situ hybridization (ISH), a 22 base pair (bp) digoxigenin-labelled DNA probe (5′ Dig-TCTCTG AGGTATGGCCGTAACT 3′) was used to detect DNA from *Pneumocystis* spp. in pulmonary and mediastinal tissues from ferrets 1 and 3, as previously described (Rosell *et al.*, 1999). Controls included tissues known to be negative and positive for *Pneumocystis* spp.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) was used to examine the pleural exudate obtained from ferret 2 and the formalin-fixed lung and lymph node from ferrets 1 and 3. After evaluating the stained slides, areas of the embedded formalin-fixed tissue where bacterial colonies were most abundant were selected for TEM. Pleural exudate was fixed with 2.5% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde (EM grade, TAAB Laboratories, Berkshire, UK) in 100 mM phosphate buffered saline (PBS, pH 7.4) for 2 h and rinsed four times with 100 mM PBS.