J. Comp. Path. 2013, Vol. 148, 419–423

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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Otitis Media Associated with *Cryptosporidium baileyi* in a Saker Falcon (*Falco cherrug*)

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Summary

A 7-week-old male Saker falcon died with a history of severe refractory dyspnoea and respiratory signs. Microscopical lesions included moderate to severe lymphoplasmacytic inflammation of the middle ears, conjunctivae, third eyelids, choanae, salivary glands of the tongue, turbinates, larynx, trachea, syrinx and bronchi. The lesions were associated with variable numbers of *Cryptosporidium* spp., further confirmed by transmission electron microscopy and in-situ hybridization. *Cryptosporidium baileyi* was identified by DNA sequence analysis. *C. baileyi* may therefore be a cause of otitis media in raptors as it is in man. It is most likely that the middle ears of the Saker falcon acquired the infection through the eustachian tubes that originate near the pharynx in the oral cavity. This is the first description of otitis media associated with *C. baileyi* in a bird or a mammal except man.

Keywords: Cryptosporidium baileyi; middle ear; otitis; Saker falcon

Cryptosporidium spp. are coccidian parasites that develop in an intracellular location at the apical surface of epithelial cells. Cryptosporidia are usually found in neonatal and young domesticated, caged and wild birds (McDougald, 2008). As primary pathogens, *Cryptosporidium baileyi* and *Cryptosporidium meleagridis* cause respiratory and intestinal disease, respectively. In chickens, turkeys and geese, the bursa of Fabricius and conjunctivae are the preferred sites of infection for *C. baileyi* (Chvala *et al*., 2006; Ryan, 2010). In turkeys and quail, *C. meleagridis* causes small intestinal infection associated with diarrhoea. In addition to the three recognized avian species, also including *Cryptosporidium galli*, a total of 10 novel genotypes have been identified in birds (Ryan, 2010). The first confirmed cases of *C. baileyi* infection in a bird species from the order Falconiformes were described recently in two captive falcons with rhinitis, tracheitis and mild conjunctivitis (Rodriguez-Barbon and Forbes, 2007) and in three mixed-bred falcons suffering from upper respiratory diseases characterized by epiglottal swelling and dyspnoea (van Zeeland *et al*., 2008). In wild raptors, an ocular and respiratory disease has been associated with *C. baileyi* in captive scops owls (Molina-Lopez *et al*., 2010). *Cryptosporidium* spp. in birds usually colonize the respiratory tract, conjunctivae, gastrointestinal tract, urinary tract, salivary glands and bursa of Fabricius, but the organisms have not been detected in the middle ears of any animal species except man. In fact, the only report of cryptosporidia in the middle ear was in a 42-year-old man suffering from a 5-year history of otitis associated with human immunodeficiency virus (HIV) infection (Dunand *et al*., 1997). The present report describes *C. baileyi*-associated otitis media and

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2012.09.005

respiratory disease in a Saker falcon (*Falco cherrug*), confirmed by transmission electron microscopy (TEM), in-situ hybridization (ISH) and DNA sequence analysis.

A falconer reported respiratory signs and death in 10 out of 15 Saker falcons and two out of 11 peregrine falcons. The birds were all 5–7 weeks of age. A 7-week-old male Saker falcon, which was resistant to treatment with antifungal drugs, antibiotics or nebulization with oxygen, was submitted for post-mortem examination to the California Animal Health and Food Safety Laboratory System (CAHFS), Fresno Branch, California, USA. The bird had a history of a progressively worsening dyspnoea, cyanosis of the face and wet gurgling over the past 2–3 weeks. Haematological and serum biochemical examinations revealed a mildly decreased haematocrit (40%; normal range 42–53%), mildly elevated phosphorus (2.29 mmol/l; range 0.29–1.84 mmol/l) and calcium (2.72 mmol/l; range 1–2.5 mmol/l) and reduced glucose (14.87 mmol/l; range 16.98–31.86 mmol/l) and aspartate aminotransferase (103 U/l; range 182–761 U/l). The birds were fed quail and water *ad libitum*.

At necropsy examination, the animal was well preserved, mildly dehydrated, emaciated and weighed 700 g. The tracheal mucosa was effaced by a diffuse grey discolouration and there was an increased quantity of mucus in the lumen. The lung was moderately congested and oedematous and the abdominal air sacs were mildly cloudy. The liver and spleen were mildly enlarged, and the latter organ was mottled pale and red. The pectoral muscles were moderately reduced in size.

Tissue samples were fixed in 10% neutral buffered formalin and processed routinely. Sections were stained with haematoxylin and eosin (HE), periodic acid–Schiff (PAS) and Kinyuon Ziehl Neelsen (KnZN). On histological examination, moderate to severe lymphoplasmacytic inflammation associated with a variable number of *Cryptosporidium* spp. was observed in the salivary glands of the tongue, in the choanae, turbinates, bulbar conjunctivae and third eyelids, larynx, trachea, syrinx and the primary, secondary and tertiary bronchi. Similar inflammation effaced the mucosa and submucosa of both middle ears, with occasional lymphoid nodule formation. Moreover, middle ears contained moderate to large amounts of intraluminal exudate composed of inflammatory cells including multinucleated giant cells mixed with fibrin, cell debris and sloughed epithelial cells (Fig. 1). Attached to the apical surface of both intact and sloughed mucosal epithelial cells were numerous PAS- and KnZN-positive round bodies, 4–6 μm in diameter, consistent with *Cryptosporidium* spp. (Fig. 2). No cryptosporidia were observed in the bursa of Fabricius, gastrointestinal tract or urinary tract. Other lesions included occasional multifocal giant cell granulomas surrounding central necrosis in the liver, increased mononuclear phagocytes in the spleen and mild squamous metaplasia of the submucosal oesophageal glands due to vitamin A deficiency, as confirmed by low vitamin A content in the liver.

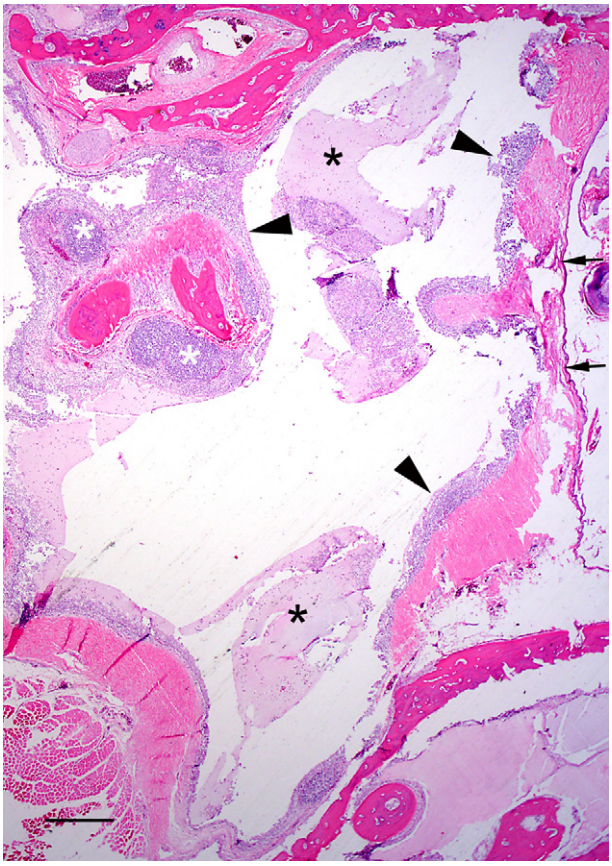

Fig. 1. *Cryptosporidium baileyi*-induced otitis media in a Saker falcon. Exudate (black asterisks) within the middle ear cavity, mucosal and submucosal inflammation (arrowheads) and occasional lymphoid follicles (white asterisks). Normal tympanic membrane (arrows). HE. Bar, 500 μm.

A previously described procedure (Chvala *et al.*, 2006) was applied to identify *Cryptosporidium*-specific 18S ribosomal RNA (rRNA) sequences in paraffin wax-embedded tissues by means of ISH. The probe sequence (5′-GTGCTGAAGGAGTAAGGAACAA CCTCCAATCTCTAGTTGG-3′) was complementary to a segment of 18S rRNA of all *Cryptosporidium* species and had sufficient nucleotide differences from other related protozoa of the phylum Apicomplexa. ISH demonstrated many dark brown protozoal bodies attached to the apical surface of the epithelial cells of the middle ear (Fig. 3). *Cryptosporidium* spp. was also confirmed in the salivary glands and the other respiratory organs.