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Evidence-based practice

Citrobacter freundii infection in two captive Australian king parrots (*Alisterus scapularis*)

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Abstract
An adult and a juvenile male Australian king parrot (*Alisterus scapularis*) were found dead in their facilities in a private aviary in June 2010 without premonitory clinical signs before death. Gross examinations showed enlarged livers, enlarged spleens with petechiae, distended proventriculi, and distended and haemorrhagic intestinal tracts. Unilateral pneumonia and enlarged kidneys were also observed in the adult parrot. Tissue samples from the heart, lungs, liver, spleen, kidneys, proventriculus and intestine were analysed using real-time polymerase chain reaction, histopathological, and bacteriological studies. *Citrobacter freundii* was isolated from several organs in the two parrots. To the authors' knowledge, this is the first report of a *C. freundii* infection causing lesions and sudden death in Psittaciformes.

Short communication

Members of the genus *Citrobacter* are gram-negative, facultative aerobic, non-sporoforming, rod-shaped bacteria belonging to the family Enterobacteriaceae (Borenshtein and Schauer 2006; Barnes and Nolan 2008). They are found in water, soil, sewage, food, faeces and urine (Borenshtein and Schauer 2006; Ocholi et al. 1988). *Citrobacter freundii* is the most pathogenic species of the genus (Gerlach, 1994) and is a ubiquitous and opportunistic animal and human pathogen (Borenshtein and Schauer, 2006; Fernández et al., 2011). *Citrobacter freundii* infection has been described in domestic, captive, and wild terrestrial and aquatic mammals, chelonians, amphibians and farmed fish (Ocholi et al., 1988, 1989; Wright and Whitaker, 2001; Galarneau et al., 2003; Jeremić et al., 2003; Steele et al., 2005; Mader, 2006; Fernández et al., 2011).

Citrobacter freundii is highly pathogenic in young and immunosuppressed avian hosts, causing septicaemia and hepatitis (Godoy and Matushima 2010), and has been isolated in Galliformes (Barnes and Nolan 2008), Anseriformes (Barnes and Nolan 2008), Passeriformes (Gerlach 1994; Godoy and Matushima 2010), Struthioniformes (Gerlach 1994), Charadriiformes (Steele et al. 2005) and Podicipediformes (Steele et al. 2005).

In June 2010, a juvenile male and an unrelated adult male Australian king parrot (*Alisterus scapularis*) were submitted from a private aviary located in La Plata City (Buenos Aires Province, Argentina) to the Laboratorio de Diagnóstico de las Enfermedades de las Aves y los Pilíferos (Cátedra de Patología de Aves y Pilíferos, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata) for postmortem evaluation. The aviary contained South American, African and Australian psittacine and passerine birds, which were kept in outdoor and indoor facilities.

The king parrots were maintained in outdoor cages without any enrichment. Their diet consisted of a commercially available seed mixture, fruits and vegetables. Neither parrot showed evidence of clinical signs before death, and they were immediately submitted under refrigeration for study.

At necropsy, both carcasses showed evidence of poor nutritional condition. Enlarged livers, enlarged spleens with petechiae, distended proventriculi, haemorrhagic content in the small intestine and distended intestinal tracts due to gas content were noticed. Additionally, unilateral pneumonia and enlarged kidneys were observed in the adult bird. Tissue samples from the heart, lungs, proventriculus, intestine, liver, spleen and kidneys were fixed in 10% buffered formalin for 48 h, embedded in paraffin, sectioned at 3 µm, and stained with hematoxylin and eosin. Tissue samples from the heart, lungs, liver, spleen, intestine and kidneys were analysed for bacteriology, inoculated onto MacConkey's agar (Laboratorios Britania, S.A., Buenos Aires, Argentina) and 10% defibrinated

horse blood agar (Laboratorios Britania, Buenos Aires, Argentina) and incubated aerobically for 24 h at 37°C.

A molecular study involving the detection of the DNA of viral pathogens of Psittaciformes, such as psittacine adenovirus, psittacine herpesvirus, psittacine beak and feather disease virus and avian polyomavirus, was also performed. Frozen tissue samples were analysed using a commercially available DNA extraction kit (DNeasy® Blood & Tissue Kit, QIAGEN GmbH, Hilden, Düsseldorf, 40724, Germany) according to the manufacturer's instructions. A polymerase chain reaction (PCR) assay was carried out with the primers and programme previously reported by Katoh et al. (2008). Amplification and detection were carried out with an IQTM5 Multicolor Real-Time PCR Detection System (BIO-RAD Laboratories, Hercules, California, CA 94547, USA). The real-time PCR mixtures were prepared with 12.5 µl of SYBR Green (IQTM SYBR® Green Supermix, BIO-RAD Laboratories, Hercules, California, CA 94547, USA), 1 µl of each primer, 7.5 µl of nuclease-free-water, and 3 µl of DNA extract made up to 25 µl of mixture.

The histopathological study revealed multifocal necrotic areas in livers and spleens and fibrinous exudate within the lumen of parabronchi and air capillaries of lungs. Mononuclear inflammatory cell infiltration and hypertrophy and hyperplasia of pneumocytes were also noted. Gram-negative bacterial colonies were found in blood vessels of liver and spleen in both specimens. The bacterium isolated in Mac Conkey's agar from several organs such as heart, lungs, liver and spleen of both parrots was identified as *Citrobacter freundii* following standard procedures (Borenshtein and Schauer, 2006). Neither bacterial infection other than *C. freundii* nor parasitic infections were detected. Psittacine herpesvirus, psittacine adenovirus, psittacine beak and feather disease virus, and avian polyomavirus are known to cause fatal illnesses in psittacine birds and to predispose them to secondary infections (Katoh et al. 2008), but none were detected in the tissue samples analysed in this study. The microscopic findings were similar to those previously reported in an epizootic infection of *C. freundii* in a colony of guinea pigs (Ocholi et al. 1988). Although no anaerobic culture analysis was carried out, the gross findings, the histopathological study, and the bacteriological isolation and identification led to the diagnosis of *C. freundii* infection in these Australian king parrots. Since to the authors' knowledge there are no previous reports describing *C. freundii* infection in parrots, this is the first description of *C. freundii* infection causing gross and microscopic lesions and sudden death in parrots.

Animals stressed by illness, capture and captivity are more likely to shed potentially pathogenic bacteria than healthy, free-ranging birds (Steele et al. 2005). The effects of captivity and poor nutritional conditions were identified as potential factors which could have triggered the opportunistic bacterial infection observed. Since rapid bacteraemia can occur when *C. freundii* penetrates into the intestinal mucosa (Godoy and Matushima 2010), it is supposed that the intestinal tract could be the initial site of bacteremia. Although the source of infection remains unknown, it is possible that other captive birds, feral birds and/or aviary keepers could be carriers of the causing agent and spread the infection. Further epidemiological surveillance studies will be useful to establish bacterial prevalence among the birds in this collection.

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