Diarrhoea and Death in a Cockatiel Associated With a *Clostridium difficile*-like Organism

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Case report

'Princess', a 6-month old female cockatiel, had been well from the time she had been acquired from a pet shop in February 2007 until 1/6/07 when she became quiet, lost her appetite and started to show diarrhoea. She had been fed a predominantly seed diet and did not like vegetables or fruit. Commercial pellets had not being offered. There were two other birds in the household and they were kept in separate cages from this bird.

Princess was presented at our clinic at 7 pm on 7/6/07. On physical examination she was very weak, fluffed, emaciated (60.2g body weight) and passing liquid faeces with haematochezia. Initial differential diagnoses included heavy metal toxicity, coccidiosis, worm parasites, bacterial infection, flagellates, chlamydophilosis, another toxicity or other systemic disease. She was admitted to our clinic, placed in an incubator at 27°C and given 1.5 ml Hartman's solution subcutaneously.

Initial diagnostics included a resting standing radiograph to check for heavy metal particles, which was negative. A faecal wet preparation showed, along with other bacteria, that there were 1-2 large motile rods with large terminal endospores per high-powered field. These organisms had the appearance of "dodgem cars" or tennis rackets with the handle end manoeuvring around the surrounding particulate matter. The organisms were Gram negative and comprised about 20% of the bacterial population. Also in the Gram stain were mixed Gram-positive bacteria (~65%), occasional *Candida* sp, and approximately 15% mixed Gram-negative rods without spores.

Princess was crop fed and treated with amoxicillin and clavulonic acid (100 mg/kg, orally), metronidazole benzoate (30 mg/kg, orally) and itraconazole (10 mg/kg, orally), but died overnight.

The necropsy was performed 20 hours after death. Findings included emaciation, rounded, full-thickness protrusions of the proventriculus and isthmus walls and liquid contents of the lower intestine. Wet preparation examination of the small intestine contents under high power showed occasional *Giardia* sp, inflammatory cells, red blood cells and large, non-motile rods with terminal spores corresponding to the motile organisms seen on faecal examination prior to death. *Giardia* sp was not seen in wet preparations of the large intestinal or cloacal contents.

Faeces were submitted for culture. A *Penicillium* sp was grown but no organism corresponding to the rods with the terminal spores.

Giemsa stain of the faecal smear showed a moderate number of sloughed epithelial cells often in small sheets, singly or dispersed, numerous bacteria, mostly rod-shaped (~60%) with fewer (~40%) coccoid organisms. There was a significant proportion of the rod shaped organisms that contained large oval endospores at one end giving the organisms a 'tennis racket' appearance, consistent with *Clostridium* sp. Fungal hyphae, yeasts (including *Saccharomyces*) or parasite ova were not seen.

Histologicallyt the proventriculus was somewhat autolysed with sloughing of most of the superficial epithelial cells. There were large numbers of yeasts amongst the sloughed cells, debris and seeds in the lumen. No inflammation in the proventricular wall was seen. In the kidney there were multiple small foci of lymphocytes and plasma cells in the interstitial tissue. Sections of the duodenum and large intestine were moderately autolysed with sloughing of the superficial epithelial cells but cellular and nuclear detail was still quite good. Part of the small intestine was markedly autolysed with almost complete loss of any cellular and nuclear detail of the full thickness of the intestine. Numerous bacteria, mostly rod-shaped organisms, could be seen in the lumen. There was also brown pigment in the lumen suggestive of antemortem haemorrhage or diapedesis into the gut lumen. No significant lesions were seen in other organs examined.

A final diagnosis of mild, multifocal, chronic, lymphoplasmacytic interstitial nephritis and a moderate to marked autolysis of the intestines with probable antemortem haemorrhage and possible necrosis was made.

While autolysis of the intestines hampered assessment there was evidence of haemorrhage or at least blood leakage into the lumen of the small intestine. This finding taken together with the faecal smear and clinical findings were highly suggestive of clostridial endotoxin disease. The cause of the mild interstitial nephritis was not determined.

Discussion

Helminth parasites and coccidia can cause diarrhoea in young cockatiels but these were unlikely in this patient as there was no evidence of them on faecal wet preparations or in the post-mortem examinations. Heavy metal toxicity is another common cause of diarrhoea with blood in cockatiels but radiographs of this bird did not show heavy metal particles. Candidiasis is another possible cause of diarrhoea in young cockatiels and *Candida* was identified in low numbers in the initial Gram stain and in histological sections of the proventriculus, but not other sections of the intestine. While possibly contributing to the patient's death, it is unlikely that *Candida* was the primary cause. The Gram negative 'tennis racket' shaped organisms seen on initial Gram stain were puzzling to us initially as Gram negative bacteria do not typically form endospores. The possibility that the organisms could have been a budding yeast (such as *Saccharomyces*) was considered but none of the yeasts are motile and the organisms seen did not correspond to images of common yeasts. Only a *Penicillium* sp was grown on fungal culture, it was considered non-pathogenic.

Numerous bacteria and flagellate organisms have been associated with diarrhoea in young cockatiels. *Giardia,* a common cause of diarrhoea in cockatiels, was found in low numbers in this patient. In our experience, however, on its own, *Giardia* tends to cause intermittent 'cow plop' droppings rather than liquid diarrhoea with blood and cockatiels rarely die from uncomplicated giardiasis.

At this clinic, in a faecal Gram stain on a normal cockatiel we would typically expect to see mixed Gram positive rods and cocci, an occasional *Candida* and less than 20% Gram negative rods. The faecal Gram stain for this patient fell within this pattern but also had the unusual finding of about 20% Gram-negative bacteria with terminal endospores. In the same Gram stained slide there were other bacteria that were strongly Gram positive so it did not appear that over decolourisation of the slide was a problem.

Clostridia, while typically Gram positive, are known to stain Gram negative under some conditions. *Clostridium difficile, C. tertium, C. tetani and C. tetanomorphum* are four species of clostridia that have terminal endospores, are motile and can be Gram negative. There are also a range of motile, terminal endospore forming *Bacillus* species that are typically Gram positive but could stain Gram negative under some circumstances. These include: *B. coagulans, B. pantothenticus, B. alvei, B. Brevis, B. circulans, B. macerans, B. polymyxa, B. sphericus* and *Bacilli* Wolf and Barker Groups 1,2 and 3. However, these *Bacilli* species are generally less pathogenic than *Clostridium.*

Clostridium-associated enteritis is a recognised entity that is seen from time to time in this clinic, particularly in lorikeets.[1] On Gram stain these birds generally show large numbers of Gram-positive rods. Endospores are not typically seen. Haemorrhagic enteritis associated with *Clostridium perfringens* is a well recognised disease in poultry and waterfowl, however *C. perfringens* has sub-terminal spores, which was not the case in the bacteria described here. Clostridia are generally susceptible to penicillins and metronidazole and this treatment was initiated with this bird. While the antibiotic may kill the bacteria it does not neutralise endotoxin. Unfortunately the bird was close to death when the treatment started and it was not successful.

The identity of the organism in question in this patient could not be determined with certainty because we were not able to culture it. Morphologically, however, the large terminal spores closely resembled images of *Clostridium difficile. Clostridium difficile* is a Gram positive, anaerobic, spore forming, motile rod-shaped bacterium that commonly inhabits the intestinal tracts of birds.[2] This organism is difficult to culture and has been reported to cause diarrhoea and death in a range of species, including birds. In humans it is responsible for approximately 3 million cases of diarrhoea and colitis annually with a mortality rate of 1 to 2.5 %. Major predisposing factors for disease in humans include antibiotic therapy, advanced age, multiple severe underlying disease and faulty immune response to *C. difficle* toxins A and B. Metronidazole is recommended as first line treatment with vancomycin an expensive alternative in resistant cases.[3,4]

There is no proof that this *Clostridium difficile*-like organism was the cause of death in this patient. It is likely that concurrent giardiasis and yeast infection would have also contributed her demise. However, the gastrointestinal bleeding or blood leakage into the intestinal lumen

was consistent with clostridial endotoxin disease. Practitioners should be alert for the characteristic large and sometimes motile rods with terminal endospores of *C. difficile* on faecal wet preparations, and be aware that some species of *Clostridium* can sometimes be Gram negative.

References

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