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Treatment of Otitis Externa Associated With *Corynebacterium kroppenstedtii* in a Peach-Faced Lovebird (*Agapornis roseicollis*) With an Acetic and Boric Acid Commercial Solution

An Martel, DVM, PhD, Freddy Haesebrouck, DVM, PhD, Tom Hellebuyck, DVM, and Frank Pasmans, DVM, PhD

Abstract: A 5-year-old lovebird (*Agapornis roseicollis*) was presented with scaly crusts around both external ear openings and exudate present around the left ear. The bird had been treated with ivermectin and enrofloxacin without success. A pure culture of *Corynebacterium kroppenstedtii* was isolated from both ears. After susceptibility testing, a treatment of an acetic and boric acid solution administered topically 3 times daily was prescribed. The scaly appearance disappeared after 14 days of treatment and *C kroppenstedtii* could not be reisolated.

Key words: *Corynebacterium kroppenstedtii*, otitis externa, acetic and boric acid solution, avian, peach-faced lovebird, *Agapornis roseicollis*

Clinical Report

A 5-year-old male peach-faced lovebird (*Agapornis roseicollis*) weighing 43 g was referred to the Clinic of Avian and Exotic Animal Diseases for evaluation of crusty lesions around both ear openings (Fig 1). The lesions had been present for 2 months and the referring veterinarian had treated the bird with ivermectin (4 doses, 0.4 mg/kg SC, q 7 d; Ivomec, Merial, Brussels, Belgium) and enrofloxacin (100 mg/L drinking water, for 4 weeks; Baytril 10%, Bayer, Leverkusen, Germany) with no improvement. The bird was housed alone and was on a pellet and seed-based diet supplemented with fruits, vegetables, and yogurt.

On physical examination, the bird was alert and had adequate body condition. The skin around both external ear openings appeared scaly with absence of feathers in this area. Exudate was visible around the left ear. No parasites were observed on examination of skin scrapings, and cytologic results revealed the presence of irregular gram-positive bacteria. The owners declined blood tests. Swabs from both ears were submitted

for bacterial (aerobic, microaerophilic, and anaerobic) and fungal culture. A pure culture of *Corynebacterium kroppenstedtii* was isolated from swabs from both ears. The bacterium was grown for 48 hours on Columbia agar with 5% sheep blood (Oxoid, Basingstoke, UK) at 37°C and 5% CO₂ atmosphere. The bacteria were non-spore-forming and nonmotile. The biochemical profile of the isolate was determined by the API Coryne system (API, Marcy L'Etoile, France) but was not recognized by the database. For further identification, the 16S rRNA gene was sequenced and sequences were compared with those maintained in the GenBank database through BLAST.¹ Disk diffusion tests revealed antibiotic resistance only to enrofloxacin and susceptibility to lincomycin, clindamycin, erythromycin, tylosin, tetracycline, penicillin, neomycin, oxacillin, gentamicin, trimethoprim sulphonamides, ceftiofur, and amoxicillin clavulanic acid.

A 2% acetic acid and 2% boric acid aqueous solution (Malacetic Otic, Eurovet, Heusden-Zolder, Belgium) is commercially available as an ear and skin cleaner for use in dogs. To determine possible efficacy of this product in this case, the bactericidal effect of 3 different dilutions of this solution on the *C kroppenstedtii* strain was determined.² Approximately 5×10^7 colony-forming units (cfu) of the *C kroppenstedtii* isolate

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Figure 1. Scaly crusts around the external ear opening of a peach-faced lovebird.

were suspended in 5 ml of undiluted, one-half dilution (1% and 1%), and one-fourth dilution (0.5% and 0.5%) of the acetic acid-boric acid solution. Dilutions were made with phosphate-buffered saline. As a control, approximately 5×10^7 cfu of the *C. kroppenstedtii* isolate were suspended in 5 ml distilled water. All suspensions were incubated at 37°C. At 0, 5, 10, 15, and 30 minutes, 3 samples of 60 μ l were collected, and the bacterial titer (number of cfu/ml) in these samples was determined by plating 10-fold dilutions on Columbia agar with 5% sheep blood. This bactericidal assay was done 3 times, and

results are summarized in Figure 2. After 10 minutes of incubation, *C. kroppenstedtii* was inactivated by the 2% acetic and 2% boric acid aqueous solution (undiluted) and had decreased numbers of cfu/ml at the one-half (1%) and one-quarter (0.5%) dilutions (Fig 2). Therefore, topical treatment with the 2% acetic acid and 2% boric acid solution was started. The solution was applied 3 times daily on the lesions with a cotton swab. After 14 days, the crusts were resolved and the skin appeared normal. Culture results of a swab sample of both external ear openings taken 7 days after the treatment was finished was negative for bacterial growth.

Discussion

External otitis in birds has been associated with various pathogens including bacteria, viruses, fungi, and parasites. Mild to severe inflammation of the external ear associated with bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pasteurella multocida*, *Proteus mirabilis*, and *Enterococcus* species has been described.³ To our knowledge, this is the first description of *C. kroppenstedtii* as an avian pathogen.

Corynebacteria have been isolated from a wide range of environments (eg, dairy products, soil, sewage, sediments, aquatic sources, and animals), but they can also occur as part of the indigenous

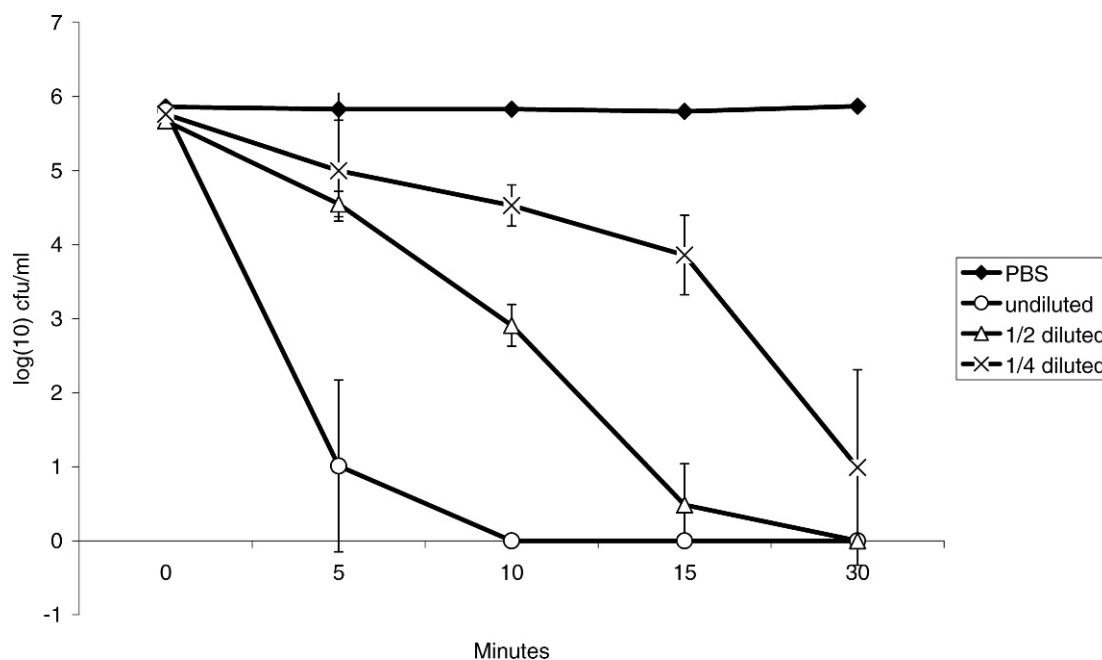


Figure 2. Logarithmic mean and standard deviation of the number of colony-forming units (log10 cfu) per milliliter for *Corynebacterium kroppenstedtii* after incubation at 37°C in phosphate-buffered saline (PBS), and in an undiluted, one-half diluted, and one-fourth diluted aqueous 2% acetic acid and 2% boric acid solution.

microflora of animals and man.⁴ *Corynebacterium falsenii*, *Corynebacterium aquilae*, *Corynebacterium sphenisci*, *Corynebacterium ciconiae*, *Corynebacterium spheniscorum*, and unidentified *Corynebacterium* species have been described as resident microbiota in different species of birds.^{5–11} Although corynebacteria are considered normal microflora, the ability of some of these bacteria to cause disease in humans and animals is well established. The only clinical case described in birds is a report on *Corynebacterium endophthalmitis* and osteomyelitis in a great horned owl (*Bubo virginianus*).¹²

Corynebacterium kroppenstedtii was first described in 1998 after the isolation of a single strain from a sputum sample from a woman with chest infection and is occasionally recognized in human clinical samples from breast tissue, pus, or deep wound swabs of patients with mastitis.^{13–16} To our knowledge, no isolations from animals have been described. Because of its association with infections in humans, the possible zoonotic transfer from an infected bird to humans should be considered. The source of the bacteria in this case is unknown.

The susceptibility of *C. kroppenstedtii* to a large panel of antimicrobial agents has never been reported. The strain isolated by Riegel et al.¹⁶ from a breast abscess in a woman was sensitive to amoxicillin, cefotaxime, rifampin, erythromycin, pristinamycin, linezolid, and vancomycin, but was resistant to fosfomycin. Our strain was susceptible to lincomycin, clindamycin, erythromycin, tylosin, tetracycline, penicillin, neomycin, oxacillin, gentamicin, trimethoprim sulphonamides, ceftiofur, and amoxicillin clavulanic acid but was resistant to enrofloxacin. This may explain why the oral treatment with this drug prescribed by the referring veterinarian was unsuccessful. It is not known if the fluoroquinolone resistance was acquired or if the bacterium is intrinsically resistant. Also, no information is available about the concentration of enrofloxacin in the skin of this animal.

Alternatives to antimicrobial treatment are sought for in all animal species, including man, because of increasing levels of acquired antimicrobial resistance and the profound effects of systematic antimicrobial treatment to the animal's bacterial communities. As an alternative to antimicrobial therapy, acetic and boric acids have been suggested for local treatment of bacterial infections. Both acids have been shown to exert antibacterial effects on different bacterial species, including staphylococci.^{2,17,18} A 2% acetic acid and 2% boric acid aqueous solution is commercially available as an ear and skin cleaner for use in

dogs. In the present study, a bactericidal effect of an aqueous solution containing at least 0.5% acetic acid and 0.5% boric acid against *C. kroppenstedtii* was found in vitro. In vivo treatment resulted in clearing the infection.

In this report, in vitro activity of the substance tested correlated well with in vivo improvement of lesions and resolution of the bacterial infections. This might be attributed at least in part to the high local concentrations reached by topical application.

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