Isolation of *Acinetobacter lwoffii* from a Lovebird (*Agapornis roseicollis*) with severe respiratory symptoms

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Summary

Although *Acinetobacter lwoffii* is generally considered an ubiquitous and opportunistic bacterium, this germ has been isolated from the pulmonary and abdominal air sac swabs obtained from a Lovebird *(Agapornis roseicollis)*, which died of a severe respiratory disease. Bacteriological tests (phenotypic and genotypic) led to the identification of *A. lwoffii* in pure culture. All the other parrots in the breeding centre were treated orally with oxytetracycline for 14 days and 3 months later no bird showed any signs of respiratory symptoms.

Keywords: *Acinetobacter lwoffii*, *Agapornis roseicollis,* isolation, respiratory disease

Isolierung von *Acinetobacter lwoffii* bei einem Rosenköpfchen (*Agapornis roseicollis*) mit schwerer Atemnot

Obwohl *Acinetobacter lwoffii* als ein ubiquitär vorkommendes und opportunistisches Bakterium gilt, gelang es, diesen Keim mittels Tupferproben aus dem Brust- und Bauchluftsack eines Rosenköpfchens (*Agapornis roseicollis*), das an schwerer Atemnot verstorben war, zu isolieren. Mit Hilfe von bakteriologischen Tests (Phänotyp und Genotyp) liess sich *A. lwoffii* in Reinkultur nachweisen. Alle übrigen Papageien der Zuchtstation wurden während 14 Tagen mit Oxytetracyklin behandelt, und 3 Monate später zeigte keiner der Vögel Anzeichen einer Atemwegserkrankung.

Schlüsselwörter: *Acinetobacter lwoffii*, *Agapornis roseicollis,* Isolierung, Respirations-Pathologie

Introduction

Acinetobacter species are usually considered non pathogenic bacteria for healthy humans and animals, and they represent normal germs of the microbial flora. In human medicine, however, they have been implicated in hospital-born infections in debilitated individuals (Euzéby, 2003). Risk factors contributing to reduced patient resistance include malignancy, burns, immunosuppression and major surgery. Occasionally, sporadic cases seem to occur in healthy individuals exposed to environmental sources (Rathinavelu et al., 2003). The major genomic species associated with outbreaks of nosocomial infection is *A. baumannii,* followed by *Acinetobacter* genomospecies 3, *A. johnsonii,* and *A. lwoffii* (Bergogne-Bérézin and Towner, 1996; Bouvet and Grimont, 1987). *A. lwoffii* is normally present on skin, oro-pharynx and perineum in about 20–25% of healthy individuals. Moreover, *A. lwoffii* has been found to be responsible for various types of opportunistic infections including endocarditis, gastritis, pseudomeningitis (Valero et al., 1999; Gusten et al., 2000; Rathinavelu et al., 2003).

In veterinary medicine *Acinetobater* spp. has recently been isolated in dogs with tracheal collapse (Johnson and Fales, 2001), in horses with intravenous jugular catheters (Vaneechoutte et al., 2000), in the respiratory system of symptomatic foals (Boguta et al., 2002), in the conjunctiva of healthy elephants (Tuntivanich et al., 2002) and also in water, aerosols and air, in poultry abattoirs (Fries and Graw, 1999). To the best of our knowledge, only a few cases of *A. lwoffii* has been reported in bird specimens, in particular in the lungs of two hens with septicemia (Kaya et al., 1989). The isolation of *A. lwoffii* from pulmonary and abdominal air sac swabs of a Lovebird *(Agapornis roseicollis)* which died of a respiratory pathology, is reported here.

Case history

In November 2003 we investigated the death of a Lovebird *(Agapornis roseicollis)* coming from a little farm in Northern Italy, where twenty pairs of the same species were going through the reproduction period. The bird lived in an indoor facility situated in a single room (3 m × 6 m) in the presence of air draughts. The aviary included 20 suspended enclosures housing the breeding pairs and two bigger cages housing 7 and 9

 DOI 10.1024/0036-7281.147.06.267

juvenile lovebirds, respectively. The food consisted basically of seed mixture and some fresh vegetables. Food was supplied in hard plastic bowls and bottled water, which were changed and cleaned daily. A specific commercial product containing protein and calcium was added to the breeding pairs' diet. Disinfection of enclosures was made with chlorexidine solution (Sonica® CL 4%, Soltec, Milan) every six months. Diagnostic controls for Circovirus, Polyomavirus (Bert et al., in press) and *Chlamydophila psittaci* (ELISA, «*Chlamydophila psittaci* Immunocomb», Biogal, Israel) were performed, at the beginning of the breeding period in all breeding pairs. The last routine control was done in March 2003 with negative results. No preventive treatments were carried out.

A two year old male Lovebird *(Agapornis roseicollis)*, which had been dead since a few hours was brought to our facilities for post-mortem inspection. During the last four days, the bird had shown severe respiratory signs, such as nasal discharge and acute dyspnea, and had been treated with 200 mg/l enrofloxacin (Baytril® 2.5% suspension, Bayer, KS) per os in the drinking water. A few days previously, two birds housed in an aviary next to the one where the Lovebird used to live also died from respiratory disease in spite of treatment with the same antibiotic. No new birds had been introduced into the farm for at least three months before the Lovebird's death. The signs first appeared during the last week of October, following a sudden decrease in the atmospheric temperature.

Laboratory findings

The post-mortem inspection revealed good general condition and the only gross lesion consisted of severe lung congestion and inflammation. Histological examination showed areas of bacterial pneumonia, characterized by the presence of infiltration of granulocytes and bacteria in the lung parenchyma. The Giemsa stains to detect *Chlamydophila psittaci* in lung, spleen and liver tissue were negative. Furthermore, Ziehl-Neelsen stain for *Mycobacterium* species in lung tissue as well as fungal cultures were also negative.

Bacterial culture was performed in Columbia Agar with 5% sheep blood and McConkey Agar (Oxoid, Basingstoke, England). The plates were incubated at 37 °C in aerobic atmosphere for 24 h. Fungal cultures were performed on Sabouraud Dextrose Agar (BD Biosciences, Sparks, USA) at 25 °C for 72 hours. Only poor growth of microorganisms on sheep blood plates was observed. The colonies were mucoid, greyish-white, and showed slight hemolysis. Microscopic examination of bacteria showed a number of short plump gram negative rods. Colonies were identified as *Acinetobacter lwoffii* by BBL CRYSTAL™.

The cultures were tested for antimicrobial susceptibility by the Kirby-Bauer (1966) standardized disc diffusion method using discs (Oxoid, Basingstoke, England) with antibiotics: amikacin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), colistin (10 µg), enrofloxacin (5 mg), gentamicin (10 µg), netilmicin (30 µg), penicillin G (10 U), piperacillin (100 µg), tetracycline (30 µg), tobramycin (10 µg). The organisms were susceptible to gentamicin, piperacillin, amikacin, tobramycin, netilmicin, tetracycline, and cefotaxime. They were resistant to penicillin G, ceftazidime, enrofloxacin, and colistin.

In addition, a fragment of 500 bp of the 16S rRNA gene of a microrganism was sequenced and compared to known sequences of ribosomal RNA genes and alignment profiles were derived from the sequences. Our strain had a homologous sequence in the first 500 base pairs of 16S RNA gene of *A. lwoffii*, accession number AY277551, with BLAST (htpp://www.ncbi.nlm.nih.gov/blast/index.htlm).

Therapy

All parrots of the breeding centre were orally treated with 200 mg/l oxytetracycline (Terramicina® powder 5%, Pfizer, Italy) per os in the drinking water for 14 days and no other bird showed any respiratory signs for the following three months.

Discussion

Acinetobacter are naturally resistant to penicillin G, although the use of a large range of antibiotics selected multiresistant strains, among them especially *A. baumannii* (Euzeby, 2003). However, the strain which was isolated in our case was still sensitive to some of the antibiotics available, thus allowing us a suitable therapy. Although it is difficult to collect bacteriological samples from the respiratory tract in small sized birds, it is recommended to identify the infectious agent and determine the antimicrobial susceptibility in order to select the appropriate treatment.

Respiratory tract infections in birds represent an important factor of economic loss for farmers. Pathogens commonly associated with respiratory infections include strains of *Chlamydophila psittaci*, *Streptococcus* spp., *Staphylococcus* spp. and *Mycobacterium* spp. In most circumstances, although *Streptococcus* spp. and *Staphylococcus* spp., are considered as part of the normal bacte-

rial flora, these microrganisms have been associated with respiratory disease when isolated in pure culture (Tully and Harrison, 1997). *Acinetobacter* spp. are generally known to be commensal bacteria and their isolation from ill animals is generally not related to specific clinical signs. Therefore, establishing their role as pathogens is extremely complicated. In human medicine, the role of *Acinetobacter* as a primary pathogen has been determined in immunodepressed patients and in those individuals who undergo repeated antibiotic treatments, promoting antibiotic resistance.

In our case, the circumstances were indeed similar. *A. lwoffii* was isolated from a weak bird, stressed by reproduction, air draughts and temperature drop. It was treated with enrofloxacin, an antibiotic towards which the cultured germ showed resistance, resulting in increased weakness of the bird and even higher pathogenic potential of the germ. Its isolation in pure culture from the lower respiratory tract of a bird affected by acute respiratory disease and histopathological lesions suggesting bacterial pneumonia, might indicate that this bacterium played an important role in the pathogenesis of this infection.

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Received: 20 November 2004
Accepted: 10 March 2005