Megabacteria and Proventricular/ Ventricular Disease in Australian Birds.

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Introduction

Proventricular/ventricular disease (PVD) is a debilitating and economically costly chronic wasting disease of a wide range of birds both in Australia and overseas. Megabacteria are large, single celled, Gram-positive, rod-shaped organisms frequently found in large numbers in the proventriculus and ventriculus of birds with PVD. They may also be found in wet mounted or Gram-stained faecal preparations (Filippich, O'Boyle et al. 1993) of affected birds. The organisms are 2-4 µm in width and up to 50 µm in length and are cigar shaped with rounded ends and mottling. They may be straight, bent, Y shaped, arranged end to end or constricted along their length as though they have a collar. The taxonomy or pathogenicity of megabacteria are unknown, yet due to their association with PVD, they have been implicated as its cause.

Incidence

PVD and associated megabacteria infection have been reported in a range of captive birds (table 1). Megabacteria additionally have been reported in Australian wild trapped European goldfinches (*Carduelis carduelis*), sulphur crested cockatoos (*Cacatua galerita*) (Filippich and Parker 1994[1]) and galahs *(Eolophus roseicapilla)* (Macwhirter 1995).

Clinical Signs

Acute outbreaks of PVD have been reported in budgerigar colonies associated with sudden death, vomition of frank blood and the passing of dark tarry faeces (Filippich and Parker 1994[2]).

The chronic form of PVD is, however, most common. Affected birds are usually greater than one year of age and progressively lose condition, become listless, dehydrated and fluffed. They are often observed at the food bowl, mouthing and

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grinding feed yet swallowing very little. They may vomit or gag intermittently and pass bulky soft faeces that are often dark and tarry and contain pieces of incompletely ground food.

Radiographically, birds with PVD given barium sulphate by crop gavage, demonstrate delayed passage through the proventriculus, proventricular dilatation and irregularity of proventricular lining.

Pathology

At necropsy, birds with PVD show proventricular and ventricular dilation. Unmacerated seeds are a common finding in the ventriculus. Ulceration and haemorrhage are often evident at the junction between the ventriculus and the proventriculus. An impression smear of this region may demonstrate large numbers of Gram positive megabacteria and erythrocytes.

Histopathologically, birds with PVD may demonstrate dilatation of the ducts of tubular alveoli of the proventricular submucosal glands, submucosal inflammatory cell infiltration, serosal thickening, rounding of plicae and hyperaemia.

Most pathology is evident at the proventricular/ventricular junction, where scanning electronmicroscopy may show dense aggregates of striated organisms attached in parallel arrays to the proventricular mucosa by a microbial glycocalyx-like material. The mucosal epithelial cells adjacent to the organisms appear damaged and may be seen extruding into the lumen.

Pathogenesis

Birds suffering from PVD usually present to the veterinary practitioner at the end stage of the disease, either dead or dieing. The pathogenesis of the disease is poorly understood. Work is currently underway to correlate clinical signs, nutritional studies, gross, histopathological and electronmicroscopic pathology better in order to understand the pathogenesis of the disease.

Megabacteria's association with PVD

The association of megabacteria with PVD is not clear. Koch's postulate has not been fulfilled. Attempts at transmission studies have been made (Gerlach.H. 1986) albeit unsuccessfully. The organisms exist in many clinically normal birds (Filippich and Parker, 1994 [1]) and may in fact be normal commensal organisms of the avian gut flora, only becoming harmful if some other predisposing factor is present (Davis, Kenzy et al. 1981; Gerlach.H. 1986). Secondary mycosis is often observed in megabacteria associated PVD.

The Taxonomy of Megabacteria.

The taxonomy of megabacteria also is not clear. *In vivo* drug trials (Filippich and Parker 1994) have demonstrated that amphotericin B (Fungilin, 100mg/ml suspension, Bristol-Myers Squibb Pharmaceutical Pty Ltd) successfully removes megabacteria from the gut of both budgerigars and European goldfinches. In addition, nystatin (Mycostatin, 100,000 U/ml suspension, Bristol-Myers Squibb Pharmaceutical Pty Ltd) is an effective treatment in European goldfinches. Amphotericin B and nystatin are polyene macrolide antifungal agents and function through binding to sterols in the fungal cell membrane and altering its permeability. Bacteria have not been reported to contain sterols in their cell walls (Filippich and Perry 1993). If megabacteria are in fact bacteria, the mechanism of action of the polyene macrolides is unknown.

Transmission electronmicroscopy studies have shown that megabacteria have a typical bacterial structure by some workers and not by others. Tsai, Park *et al.* (1992) report that their megabacterium, a filamentous organism associated with catarrhal proventriculitis in pet birds, contained a distinct eukaryotic nucleus and hence was not a bacterium. Other workers (Filippich, O'Boyle *et al.* 1993; Van Herck, Duijser *et al.* 1984) claim that megabacteria appear bacterial on the basis of cell width, the absence of intracellular membrane bound organelles and the presence of nucleoid like areas. Recent ultrastructural studies by the author demonstrate an extensive intracellular membrane network. Intracellular membrane bound organelles, however, have been shown not to contain DNA (deoxyribonucleic acid), suggesting that the organisms are not eukaryotic.

Australian megabacteria have failed our attempts to grow using routine bacterial *in vitro* cultivation techniques, including those used repeatedly to grow megabacteria by some overseas workers (Gerlach.H. 1986; Scanlon and Graham 1990; Simpson 1992). This suggests that our megabacteria do not behave as typical bacteria and as a result the author attempted to undertake molecular biology techniques to study the organisms' genetic code for information on which domain of life they belong: *Bacteria, Archaea* or *Eucaryotae*. Specifically, the gene studied was that known to be conserved throughout all forms of life, namely that coding for small sub unit ribosomal RNA (in *Bacteria* and *Archaea* and "lower" mitochondrion-less *Eucaryotae*, 16S rRNA, in *Eucaryotae*, 18S rRNA). Such approaches have been increasingly used recently to resolve problems of identifying unculturable or difficult to culture human pathogens (Relman, Loutit et al. 1990; Relman, Schmidt et al. 1992; Arnoldi, Schluter et al. 1992). However, a great deal of difficulty was encountered in subjecting megabacteria to routine molecular biology techniques such as PCR (polymerase chain reaction) due to the thickness of the cells' walls (approx. 192 nm thick) hampering lysis and subsequent release of DNA. The megabacterial cells were successfully lysed mechanically, but DNA failed to amplify, possibly because it was irreversibly damaged during the mechanical lysis process.

Table 1.

Species	Country	References
Canaries *(Serinus canaria)*	1.The United States 2.Italy 3.The Netherlands 4.The United Kingdom	1.Hargreaves 1981; Davis, Kenzy et al. 1981 2.Tarozzi 1981 3.Van Herck, Duijser et al. 1984 4.Simpson 1992
Budgerigars *(Melopsittacus undulatus)*	1.Germany 2.The United Kingdom 3.The United States 4.Japan 5.Australia	1.Gerlach.H. 1986 2.Henderson, Gulland et al. 1988; Baker 1992 3.Scanlon and Graham 1990 4.Tsai, Park et al. 1992 5.Filippich, O'Boyle et al. 1993
Ostriches *(Struthio camelis)*	1.South Africa 2.Australia	1.Huchzermeyer, Henton et al. 1993; Huchzermeyer 1994 2.Miller and Sullivan 1994
Several species of Finches and Parrots	1.The United States 2.Japan 3.Italy 4.Australia	1.Hargreaves 1981; Davis, Kenzy et al. 1981; Anderson 1993 2.Tsai, Park et al. 1992 3.Tonelli 1993 4.Filippich, O'Boyle et al. 1993; Filippich and Parker 1994

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