Megabacteria and Proventricular Disease in Birds

LJ Filippich
Department of Companion Animal Medicine and Surgery
Veterinary Surgery, The University of Queensland
St Lucia, Qld, 4072

INTRODUCTION

Megabacteria is a large, Gram-positive, periodic acid-Schiff (PAS)-positive, rod-shaped organism that has been found in the proventriculus of several species of pet birds by a number of workers (Hargreaves 1981; Van Herck *et al*., 1984; Henderson *et al*., 1988; Scanlan and Graham 1990; Baker 1992). Electron microscopy studies have shown that it has a typical bacterial structure (Van Herck *et al*., 1984). Scanlan and Graham (1990) were able to culture the organism, using special bacteriological conditions, and found that it was a capnophilic, facultative anaerobe.

Species affected. Megabacteria has been seen in routine wet-mount faecal preparations in a number of bird species as outlined in Table 1.

Prevalence of megabacteria in the bird population is unknown, although it may be high in show budgerigars in England (Baker 1992). In one study from the Netherlands, involving a small number of canaries, 30% of birds examined were found to have megabacteria in the proventriculus (Van Herck *et al*., 1984). Over a 10-year period to 1992, megabacteria was found in the droppings of clinically normal or sick birds or in histological sections taken at necropsy in 23 budgerigar breeding colonies and 2 canary colonies in South-East Queensland, although on some occasions the origin of the budgerigars could be directly or indirectly traced back to known megabacteria positive aviaries. Table 2 shows the number of birds in three budgerigar and one canary colony that were faecal positive for megabacteria.

In addition, when the droppings from 42 imported English budgerigars, owned by 4 different Australian owners and from 2 different shipments, were examined within a few days of being released from Australian quarantine, 22 (52%) were found to be megabacteria positive.

Table 1: Faecal Megabacteria Positive Species

SCIENTIFIC NAME	COMMON NAME
Melopsittacus undulatus	Budgerigar*
Serinus canaria	Border fancy canary*
Erythrura gouldiae	Gouldian finch
Heteromunia pectoralis	Pictorella finch
Taeniopygia guttata	Zebra finch
Agapornis spp.	African lovebird
Alisterus scapularis	King parrot*
Apromictus erythropterus	Red-wing parrot
Cacatua galerita	Sulphur-crested cockatoo
Cacatua roseicapilla	Galah*
Calyptorhynchus latirostris	White-tailed black cockatoo
Cyanoramphus novaezelandiae	Red-crowned kakarikis
Neophema bourkii	Bourke's parrot*
Neophema splendida	Scarlet-chested parrot*
Polytelis alexandrae	Princess parrot*
Polytelis swainsonii	Superb parrot*
Psephotus varius	Mulga parrot

*** Birds with clinical or necropsy signs of proventricular disease**

Table 2: Prevalence of Faecal Megabacteria in Breeding Colonies

	Budgerigar colonies			Canary colony
	1	2	3*	
Total tested	25	340	487	17
Cocks	14	172	226	8
Hens	11	168	261	9
Total positive	16 (64)	92 (27)	197 (40)	8 (47)
Cocks	7 (50)	50 (29)	105 (46)	4 (50)
Hens	9 (82)	42 (25)	92 (35)	4 (44)

* **Birds under 1 year of age were not tested.**
( ) - % of total number tested.

Mode of transmission of megabacteria is unknown.

When 2 pair of adult, faecal megabacteria positive budgerigars were housed in a commercially available flat-topped, wire floored cage (34 x 44 x 52 cm) with 2 pair of adult faecal megabacteria negative birds for 14 months, the megabacteria negative birds remained consistently negative.

Breeding trials, conducted in a megabacteria positive budgerigar breeding establishment, showed that of the 41 chicks hatched and raised by 15 faecal megabacteria negative pairs, 29% were faecal megabacteria positive. When 58 eggs from faecal megabacteria positive pairs were hatched and raised by faecal megabacteria negative fosters, 46% of the chicks were found to be faecal megabacteria positive. In 6 nests where megabacteria negative fosters raised their own chick with introduced chicks or eggs from other nests, which were subsequently found to be positive, the foster's chick was negative.

PROVENTRICULAR DISEASE (PVD) IN BUDGERIGARS

PREVALENCE

The incidence of PVD in birds is unknown although it appears to be low except in show budgerigars, where in one English study, 21% of budgerigars necropsied were found to have PVD (Baker 1992). Since (18 months) monitoring budgerigar colony 1, one of the 16 megabacteria positive birds has died and at necropsy had severe PVD. Of the 22

recently imported English budgerigars which were found to be faecal megabacteria positive, 4 have subsequently died and at necropsy had severe PVD.

CLINICAL SIGNS

In the acute form of PVD, which can occur as an outbreak in a budgerigar colony, birds in good condition suddenly become severely depressed, lethargic, hypothermic and usually die within 12-24 hours. Many will regurgitate blood resulting in staining of the neck and commissures of the mouth or pass reddish, black droppings.

The chronic form of PVD is much more common and is usually first seen in birds greater than 1 year of age or just after the first breeding season. The affected birds lose condition, become depressed, hypothermic and progressively lose bodyweight despite apparent polyphagia. Although the affected birds are often seen eating at the food bowl, they invariably only mouth or grind the seed in their beaks and swallow very little so that their crops, on palpation, are usually empty. Early in the disease process, birds may regurgitate crop content which can be blood tinged or mouth gag or neck stretch in an often unsuccessful attempt to regurgitate. The droppings are softer, watery and dark green to brown/black in colour and may contain seed particles and on rare occasions undigested seed. The birds progressively become emaciated and debilitated over a number of weeks or months and then either die or slowly recover. The average age of 37 birds (20 females, 17 males) that died of PVD was 2.6 ± 1.3 years and ranged from 1-5 years. Apparently recovered birds will usually relapse several weeks or months later when stressed, for example, during moulting or breeding. Thus, such birds are of little use in a breeding program.

CLINICAL WORKUP

Wet mount preparations of vomitus or faeces usually reveal large numbers of megabacteria. In about 15% of birds with PVD at necropsy, megabacteria was not found in the droppings (Baker 1992). Barium studies on budgerigars with severe PVD show decreased alimentary sojourn and proventricular dilation. Packed cell volume, plasma biochemistry and blood-gas and acid-base determinations in 6 budgerigars with severe PVD are shown in Table 3. Haematological parameters measured in 8 budgerigars with PVD have been reported by Henderson *et al*., (1988).

NECROPSY

In the chronic form of PVD, the carcass is emaciated, the crop is invariably empty and the proventriculus distended. Seed, in severe cases, can be seen through the proventricular wall. On opening the proventriculus, a cloudy thick film covers the mucosal surface. At the distal end of the proventriculus and at the proventricular/ ventricular junction, ulceration and petechial bleeding may be seen on the mucosa causing the overlying

mucous covering to be stained black in colour. Perforation of the proventriculus can occasionally occur. The ventricular kaolin layer may appear soft and devitalised and its luminal surface may be roughened and brown in colour. In birds with proventricular bleeding, the intestines may be darkened to black in colour. Microscopic examination of proventricular scrapings usually reveal large numbers of megabacteria. Scanning electron microscopy reveals the megabacteria lie parallel to one another and extend from the mucosal surface into the proventricular lumen like finger-like projections. The pH of the proventricular fluid, when measured using pH 0-6 and pH 0-14 indicator strips (Merck, Germany) in 21 budgerigars with PVD, was found to be less than 2.5 in 17 birds and greater than 3.5 in 4 birds. Van Herck *et al*., (1984) found that the pH of the proventriculus in canaries with PVD was alkaline. Histologically, the lesion is predominantly confined to the distal end of the proventriculus and proventricular\ ventricular junction. The megabacteria are superficial and do not invade the mucosal epithelium, although in severe cases they may extend down into the lumen of the

proventricular glands.

Table 3: Blood Parameters Measured in 6 Budgerigars with PVD

Parameter	Mean	SD	Range
Packed cell volume (l/l)	0.38	0.12	0.22-0.55
Sodium (mmol/l)*	129	8.1	117-139
Potassium (mmol/l)*	3.1	0.93	2.4-4.5
Chloride (mmol/l)*	99	9.4	83-106
Magnesium (mmol/l)*	0.9	0.17	0.65-1.11
Calcium (mmol/l)*	1.55	0.114	1.36-1.64
Phosphate (mmol/l)	0.93	0.369	0.5-1.62
AST (u/l) at 37°C	206	51.8	144-276
ALT (u/l) at 37°C	7.8	3.76	1-12
GGT (u/l) at 37°C	10.8	6.07	4-22
CPK (u/l) at 37°C	596	187.6	400-906
Total bilirubin (µmol/l)	1.6	0.55	0.7-2.0
Total protein (g/l)	18.5	3.95	15-27
Albumin (g/l)	5.5	0.76	5-7
Globulin (g/l)	12.8	3.29	10-20
A/G ratio	0.45	0.05	0.4-0.5
Glucose (mmol/l)	18.3	1.73	15.7-20.9
Cholesterol (mmol/l)	3.5	1.02	2.42-4.89
Uric acid (umol/l)*	329	191.8	367-505
pH at 40°C	7.37	0.023	7.34-7.40
pCO_2 (mmHg)	33	4.8	29-40
pO_2 (mmHg)	46	11.3	28-63
Bicarbonate (mmol/l)	18.4	1.41	17-21
TCO_2 (mmol/l)	19.4	1.42	18-22
Base Excess (mmol/l)	-5.0	-1.45	-3.2 to -7.2

*** Values for 4 budgerigars**

MICROBIOLOGY

Bacteriological cultures (aerobic and anaerobic) of proventricular scrapings taken from several birds with moderate to severe PVD failed to grow any common pathogens, despite the use of several culture media. On some occasions in dead or severely debilitated clinical cases, secondary bacterial and fungal pathogens can be isolated. Attempts to grow the megabacteria by plating onto sheep or budgerigar blood agar in an atmosphere of 10% carbon dioxide, MRS agar (Oxoid) or pre-reduced media for anaerobic culture sometimes combined with micromanipulation techniques failed to grow the organism. These findings are at variance with those reported by Scanlan and Graham (1990) and Simpson (1992), who were able to grow the

megabacteria.

Virology studies, to date, on proventricular tissue or blood from birds with PVD do not support the involvement of avian poxvirus, herpes virus, Newcastle disease virus or reticuloendotheliosis virus in the aetiology of PVD.

CLINICAL THERAPEUTIC DRUG TRIALS

(a) **In-water medication trials:**

Each drug was tested in 4 faecal megabacteria positive budgerigars for 4 or 6 weeks. The drugs were made up fresh each day, except for bicarbonate and acid water which were made up twice weekly. The birds in each group were housed together in a standard wire-floored cage and their age, sex and ring number recorded. The birds were selected on the basis that they were clinically normal and preferably with low faecal megabacteria counts. The body weight of each bird, using a Cenweight mini electronic scale, was measured at the beginning and at weekly intervals throughout the trial.

Faecal megabacteria status of each bird was measured at the beginning and at weekly intervals by making a thin, uniform, wet mount preparation of the faecal component of a freshly passed dropping on a clean microscope slide and using a 22 x 22 mm coverslip and examining it under light microscope with the condenser fully extended. Using a 100x magnification the preparation was scanned and less than 5 megabacteria was designated 1+, greater than 5 but less than 144 as 2+ and greater than 144 as 3+.

Feed and water intake was measured over a 48-hour period, once or twice weekly (Table 6). The feed containers were partly covered with clear plastic hoods to reduce seed spillage and the amount of seed eaten was determined by subtracting the weight of the seed left after willowing from the seed feed. The sole source of feed fed was standard budgerigar seed mix, made up of 40% canary seed, 20% white millet, 20% japanese millet, 10% pannicum and 10% hulled oats. Inverted

water containers were used and their evaporative water loss varied from 2 to 3 ml/day.

Drugs tested (Tables 4 and 5) were amoxycillin trihydrate (1.8 g/l. Central Chemical Distributors, Pendle Hill), Trimsul (250 mg sulphadiazine and 50mg trimethroprim/l. CCD, Pendle Hill), metronidazole (2 g/l. Torgyl, Webster/MB), ketoconazole (333 mg/l. Nizoral, Janssen Cilag), sodium carbonate-bicarbonate buffered water with a pH of 9.1, unbuffered hydrochloric acid water with a pH 0.91 made up by adding 6 ml of a 30% hydrochloric acid solution to one litre of water, buffered hydrochloric acid water with a pH 1.2, chloramine (1 g/l. Halasept) and chlorhexidine (10 mg/l, Gelflex, Gelflex Laboratories).

At the end of the trial the birds were necropsied and 13 birds (36%) showed macroscopic and/or histological evidence of PVD: Amoxycillin, Bird 4 ; sulpha/trimethoprim, Birds 1, 4 ; metronidazole, Birds 1 to 4 ; bicarbonate buffered water, Bird 1 ; unbuffered acid water, Bird 4 ; chloramine, Birds 1 to 3 ; chlorhexidine, Bird 3. The average age of these 13 birds was 2 ± 1.3 years and they ranged from 1-5 years.

During the study, all but 8 of the birds, remained consistently faecal positive for megabacteria although in some birds their megabacteria status fluctuated. Eight of the

1+ megabacteria positive birds became faecal negative at least once during the study and in one bird (Bird 4, chloramine) proventricular scrapings were negative at necropsy.

(b) **Crop gavage medication trials:**

Both experimental and clinical budgerigars were used to test the efficacy of a number of drugs against megabacteria. Clinical cases were not euthanased at the end of the trial. The drugs and doses used are listed in the table. All drugs were given twice daily for 10 days and bodyweight and faecal megabacteria status recorded at the start and end of each drug trial. Some birds, predominantly the clinically owned birds, were given a number of drugs consecutively.

These results support the *in vitro* findings of Scanlan and Graham (1990) that megabacteria is resistant to sulpha/trimethroprim. Our findings that megabacteria is *in vivo* resistant to amoxycillin, both orally and intramuscularly, is at variance to that found for ampicillin *in vitro* by Scanlan and Graham (1990).

Table 4: In-Water Medication Trials (4 weeks)

Bird		Drug used											
		Amoxycillin		Trimsul		Metronidazole		Ketoconazole		Bicarbonate water		Unbuffered Acid Water	
		Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
1	Weight (g)	46	44	44	32*	44	42	42	48	42	44	40	38
	Mb† Status	2+	1+	1+	3+	3+	3+	1+	1+	3+	3+	1+	2+
2	Weight (g)	42	40	40	42	32	24	44	42	50	42	46	44
	Mb Status	1+	3+	1+	1+	2+	3+	1+	1+	3+	2+	1+	2+
3	Weight (g)	48	44	44	46	40	42	42	46	40	42	50	50
	Mb Status	1+	1+	1+	1+	2+	3+	1+	2+	1+	1+	1+	-#
4	Weight (g)	42	46	40	44	42	50	40	36	50	46	50	44
	Mb Status	2+	2+	3+	3+	3+	3+	1+	2+	1+	2+	1+	3+

***** **Died of PVD at week 3.**
# **Megabacteria positive at necropsy.**
† **Megabacteria**

Table 5: In-Water Medication Trials (6 weeks)

Bird		Drugs used					
		Buffered acid water		Chloramine		Chlorhexidine	
		Start	End	Start	End	Start	End
1	Weight (g) Mb† Status	52 1+	44 - *	30 1+	34 1+	50 1+	53 2+
2	Weight (g) Mb Status	38 1+	40 - *	46 1+	42 2+	48 2+	46 1+
3	Weight (g) Mb Status	42 1+	42 1+	48 3+	42 3+	34 3+	32# 3 +
4	Weight (g) Mb Status	40 1+	48 1+	34 1+	32 - ‡	44 +	48 - ‡

*** Megabacteria positive at necropsy. # Died of PVD at week 4.**
‡ Megabacteria negative at necropsy. † Megabacteria

Table 6: Mean Values ± SD for Seed and Medicated Water Intake Measured in Groups of 4 Budgerigars over 4 or 6 Week periods

Drug	Dose	Seed intake	Medicated water intake
Control		23.1 ± 4.23	5.1 ± 2.13
Water deprivation*		19.2 ± 2.64	0 ± 0
Amoxycillin	1.8 g/l	28.2 ± 3.11	5.2 ± 1.86
Trimsul	6.25 g/l	34.1 ± 0.56	3.1 ± 0.65
Metronidazole	2 g/l	40.7 ± 5.39	2.8 ± 0.46
Ketoconazole	333 mg/l	20.6 ± 0.56	3.2 ± 0.25
Bicarbonate water	pH 9.1	25.8 ± 0.55	2.0 ± 0.56
Unbuffered acid water	pH 0.91	18.6 ± 0.92	1.4 ± 0.51
Buffered acid water*	pH 1.2	19.8 ± 0.66	1.3 ± 0.44
Chloramine*	1 g/l	30.7 ± 3.02	3.0 ± 0.79
Chlorhexidine*	10 mg/l	31.1 ± 2.98	1.61 ± 0.13

* 6 week period

Table 7: Crop Gavage Medication Trials (10 days)

Bird (age, sex)	Drug (concentration)	Dose (ml) BID	Bodyweight (g)		Megabacteria	
			Start	End	Start	End
1, F	Ketoconazole (333 mg/l)	0.5	60	50	1+	1+
2, F		1.0	58	54	1+	1+
1, M	Metronidazole (2 g/l)	1.0	44	46	3+	3+
	Cimethidine (5 mg/ml)	0.2	46	42	3+	3+
	Itraconazole (10 mg/ml)	0.05	48	50	2+	- *
2, F		0.1	62	62	1+	1+
2, M			40	34	3+	3+
3, F	Cimethidine (5 mg/ml)	0.1	50	50	3+	3+
3, F	Carafate (20 mg/ml)	0.5	42	44	3+	3+
	Bicarbonate water	1.0	44	52	3+	3+
	Amoxycillin (1.8 g/l)	1.0	52	52	3+	3+
	Mycostatin (100,000 U/ml)	0.2	52	56	3+	1+
3, M		0.4	38	36	3+	3+
2, M	Chlorhexidine (10 mg/l)	1.0	44	40	3+	3+
	Trimsul (6.25 g/l)	1.0	36	34	2+	3+
3, F	Buffered acid water	1.0	30	34	3+	3+
0.5 M	Chloramine (1 g/l)	1.0	46	44	1+	2+
1, F	Bisolvon (5 mg/ml)	0.5	53	56	2+	1+
6, M	Pot. permanganate (200 mg/l)	0.3	50	44	1+	- †
3, M	Amoxycillin (100 mg/ml)	0.1#	42	32	3+	2+

* Megabacteria negative at necropsy. PVD present.
† No necropsy performed.
# Intramuscular injection.

REFERENCES

Baker JR (1992) Megabacteriosis in exhibition budgerigars. *Veterinary Record* **131**: 12- 14.

Hargreaves RC (1981) A fungus commonly found in the proventiculus of small birds. *Proceedings of the 30th Western Poultry Disease Conference and the 15th Poultry Health Symposium*. Cooperative Extension Service, University of California-Davis, Davis, Calif. p. 75.

Henderson GM, Gulland FMD and Hawkey CM (1988) Haematological findings in budgerigars with megabacterium and trichomonas infections associated with "going light". *Veterinary Record* **123**: 492-494.

Scanlan CM and Graham DL (1990) Characterization of a Gram-positive bacterium from the proventriculus of budgerigars (*Melopsittacus undulatus*). *Avian Diseases* **34**: 779-786.

Simpson VR (1992) Megabacteriosis in exhibition budgerigars. *Veterinary Record* **131**: 203-204.

Van Herck H, Duijser T, Zwart P, Dorrestein GM, Buitelaar M and Van Der Hage MH (1984) A bacterial proventriculitis in canaries. *Avian Pathology* **13**: 561-572.

Acknowledgment.

The findings reported in this paper have arisen from collaborative work with Dr J.A. Fuerst and Mr R. Webb (Microbiology Department), Professor P.B. Spradbrow and Miss D. O'Boyle (Veterinary Pathology Department) of The University of Queensland. The work was financed in part by the author, a grant from the Australian Bird Medicine Research Trust Fund and donations from the general public and avicultural societies.