

FAECAL WET PREPARATION AND GRAM STAIN RESULTS ON 200 PARROTS AND COCKATOOS

Pat Macwhirter, BVSc (Hons), MA, PhD, FACVSc
Burwood Bird and Animal Hospital
128 Highbury Road
Burwood, Vic, 3125

Results of faecal wet preparations and Gram stains from 200 parrots and cockatoos are reviewed. Unhealthy birds with loose droppings were statistically more likely to have >25% Gram negative rods and/or >1 budding Candida spp. per oil immersion field compared with healthy birds with normal droppings. In addition, individual birds with loose droppings showed Macrorhabdus ornithogaster, flagellates, worm eggs, coccidia and/or >95% of single types of bacteria. Faecal wet preparations and Gram stains are rapid, non-invasive, inexpensive screening tests that are useful in assessing bird health, particularly in birds with loose droppings.

Gram stain and wet preparation examination of droppings are non-invasive tests that can be done in-house and provide on-the-spot results useful in diagnosing common parrot diseases - or are they? Traditional Gram stain testing has been criticised on the Veterinary Information Network as being of little value, prompting this study on 172 parrots and 28 cockatoos during 2009 and 2010.

METHODOLOGY

Birds included in the study were not on medication in the preceding 4 weeks. All birds were examined by the author, histories taken, species recorded, droppings examined and classified in one of four groups:

1. Healthy bird, normal faeces (HN)
2. Healthy bird, loose faeces (HL)
3. Unhealthy bird, normal faeces (UN)
4. Unhealthy bird, loose faeces/diarrhoea (UL)

On physical examination, birds were classified into "healthy" (those in which no abnormalities were detected) and "unhealthy" (those in which abnormalities were detected). Fresh droppings were taken from each bird and two slides prepared and examined as follows:

1. Faecal material was mixed with a drop of saline and covered with a cover slip. The slide was systematically scanned under x10 and then further examined under x 40.
2. A Gram stain was performed by heat fixing a thin faecal smear and staining as follows:
 - a. 30 seconds crystal violet, rinse with tap water;
 - b. 30 seconds iodine, rinse with tap water;
 - c. Decolourise with acetone, rinse with tap water;
 - d. 30 seconds Safranin, rinse with tap water;
 - e. Stand to dry; and

- f. Examine under oil emersion.

Results were recorded as follows:

1. From the wet preparation: flagellates, worm eggs and coccidia were recorded using a 0-2 scale (0 if absent; 1+ if any observed while scanning; 2+ if >1 per high power field).
2. From the Gram stain (oil immersion field):
 - a. an estimate of the number of bacteria on a scale of 0-3: 0 (none); 1 (1-10 bacteria/field; 2 (11-30 bacteria/field; 3 (>30 bacteria /field);
 - b. an estimate, to the nearest 10 %, of Gram positive rods, Gram positive cocci, Gram negative rods (GNR) and Gram negative cocci. (ie a score of 20% would indicate between 15% and 25% of a particular class of bacteria);
 - c. Budding *Candida* spp recorded as 0 (absent); 1+ (1 per field); 2+ (>1 per field). Non-budding *Candida* spp were not recorded; and
 - d. *M. ornithogaster* present or absent.

RESULTS

Table 1 records the results of health and faecal status of examined birds. 83 birds were healthy with normal faeces presented for initial or annual check ups (HN); 67 were unhealthy, with signs of illness and loose faeces or diarrhoea (UL); 12 appeared healthy but had loose faeces. (HL); and 38 were unhealthy but had normal faeces (UN).

Table 1. Health and Faecal Status of Examined Birds

	Healthy	Unhealthy
Normal faeces	83 (HN)	38 (UN)
Loose faeces or diarrhoea	12 (HL)	67 (UL)

Table 2 shows the species examined. 74% comprised budgerigars or cockatiels, and 26% 19 other species.

Table 2. Species unhealthy with loose faeces or healthy with normal faeces

Species	No.	%			
		Total Birds	HN	UL	% UL
Budgerigar	59	30%	22	23	39%
Cockatiel	48	24%	20	12	23%
Rainbow Lorikeet	16	8%	3	8	50%
Galah	14	7%	7	3	21%
Eclectus Parrot	14	7%	8	4	29%
Sulphur Crested Cockatoo	10	5%	3	3	30%
Princess Parrot	6	3%	2	3	50%
Peach faced lovebird	5	3%	2	2	40%
Quaker Parrot	5	3%	4	1	20%
Green Cheeked Conure	5	3%	2	2	40%
Green winged Macaw	4	2%	2	2	50%
Indian Ringneck Parrot	3	2%	1	1	33%
Sun Conure	2	1%	2	0	0%
Long billed Corella	2	1%	1	0	0%
Umbrella Cockatoo	1	1%	1	0	0%
Red tailed Black Cockatoo	1	1%	1	0	0%
Alexandrine Parrot	1	1%	1	0	0%
Musk lorikeet	1	1%	0	1	100%
Kakariki Parrot	1	1%	0	1	100%
Blue and Gold Macaw	1	1%	1	0	0%
Crimson Rosella	1	1%	0	1	100%
TOTALS	200	100%		67	33%

Table 3. Organisms detected in Faecal Wet Preparation Examination (12 of 200 birds)

Species ↓	Condition		Faeces		Organisms Identified		
	Healthy	Unhealthy	Normal	Loose	Flagellates	Ascarids	Coccidia
Budgerigar		✓		✓			2+
Budgerigar		✓	✓			1+	
Budgerigar		✓	✓			1+	
Budgerigar	✓		✓				1+
Cockatiel		✓		✓	1+		
Cockatiel	✓			✓	1+		
Cockatiel	✓			✓		1+	
Cockatiel		✓	✓		1+		
Cockatiel	✓		✓		1+		
Cockatiel	✓		✓				
Sun Conure	✓			✓		1+	
SC Cockatoo		✓		✓	2+		

1. Flagellates (all suspected to be *Giardia* spp) were identified on wet preps in five birds: four cockatiels and a Sulphur Crested Cockatoo. In two of the cockatiels these were a coincidental finding in well birds with normal droppings.
2. Worm eggs (all Ascarids) were identified in four birds, two budgies, a Sun Conure and a cockatiel, all with loose droppings,
3. Coccidia were identified in two birds, both budgies, one well and the other unwell with loose droppings.

Presence of Budding *Candida* spp.

Eight birds had $\geq 1+$ budding *Candida* spp, none of which were HN (Table 5), compared with a 83 of 200 birds that were HN. Using a 2-tailed Fisher’s exact test, birds that had an average <1 budding yeast per oil immersion field were statistically more likely to be unhealthy and have loose droppings than birds that had <1 budding *Candida* spp per oil immersion field ($p=0.0163$). Three of the birds with $>1+$ budding *Candida* spp were rainbow lorikeets.

Table 4. Species, health and faecal status of birds with >1 budding *Candida* spp per oil immersion field (faecal Gram stain).

Species	Healthy	Unhealthy	Faeces Normal	Faeces loose	Budding <i>Candida</i> spp
Rainbow Lorikeet		✓		✓	2+
Rainbow Lorikeet		✓		✓	2+
Rainbow Lorikeet		✓		✓	2+
Princess Parrot		✓		✓	1+
Cockatiel		✓		✓	1+
Cockatiel	✓			✓	1+
Sulphur Crested Cockatoo	✓			✓	1+
Peachfaced Lovebird		✓	✓		1+

Table 5. Birds with >1 budding *Candida* spp per oil immersion field (faecal Gram stain)

Species	Healthy	Unhealthy	Faeces Normal	Faeces loose
Eclactus Parrot		✓		✓
Eclactus Parrot		✓		✓
Rainbow Lorikeet		✓		✓
Sulphur Crested		✓		✓
Long Billed Corella	✓			✓
Rainbow Lorikeet		✓	✓	
Sulphur Crested		✓	✓	
Budgerigar	✓		✓	
Budgerigar	✓		✓	
Blue and Gold Macaw	✓		✓	
Galah	✓		✓	
Rainbow Lorikeet	✓		✓	
Sulphur Crested	✓		✓	

The values for 13 birds with <1 *Candida* spp per oil immersion field (see Table 5) were less clear, with six of these being HN.

Results of Gram Stains – Bacteria

- Three birds showed > 1+ bacteria and > 95% Gram positive rods
 - ▶ one budgerigar and two rainbow lorikeets, all UL
- One bird, a budgerigar UL showed > 1+ bacteria and > 95% Gram positive cocci, one budgerigar UL.

- 36 birds from eight species showed > 1+ bacteria and > 25% Gram negative bacteria on Gram stain (Table 7).
 - ▶ Using a 2-tailed Fisher's exact test, birds with > 25% GNRs were statistically more likely to be unhealthy and have loose droppings than birds that had <25% GNRs.
 - ▶ Birds with <25% GNRs were 15.6 times more likely to be HN than birds with > 25% GNRs. If cockatiels were removed from the data set the results were even more striking.

Table 6. Health and Faecal Status of Birds with <25% Gram negative rods compared with birds with >25% Gram negative rods.

	Birds with <25% GNRs	%	Birds with >25% GNRs	%	Species
HN	78	48%	5	14%	4 cockatiels, 1 galah
HL	36	22%	2	5%	2 cockatiels)
UN	5	3%	7	19%	2 cockatiels, 2 Rainbow Lorikeets, 1 each galah, Indian Ringneck,
UL	27	27%	23	62%	11 budgerigars, 4 cockatiels, 3 Rainbow Lorikeets, 2 galahs, 1 each Eclectus Parrot, Kakariki
Total 200 birds	163	100%	37	100%	

M. ORNITHOGASTER

M. ornithogaster was identified on Gram stain in a healthy cockatiel and in a cockatiel that was unhealthy with loose droppings. In one, *M. ornithogaster* was not identified on wet preparation but was identified on Gram stain. In addition, organisms that appeared to be *M. ornithogaster* were suspected in a wet prep of a Peach-Faced lovebird but on Gram stain these appeared to be large bacteria. Gram stain appeared to be more reliable than wet preparation examination in identifying *M. ornithogaster*.

Healthy birds with normal faeces compared with birds unhealthy and with loose faeces (Table 7)

Of the 83 HN birds, flagellates were found in one cockatiel and a coccidian in one budgerigar. Six birds had budding *Candida* spp but none had >1 per oil immersion field. There were 26 birds with > 5% Gram negative bacteria, 51 birds with 5-25% GNR and 6 birds with > 25% Gram negative bacteria, four of which were cockatiels. There were no birds with >95% of any individual class of bacteria.

Table 7: HN Birds Compared with UL Birds

	HN	UL	Total
Coccidia	1	1	2
Flagellates	2	1	5
<i>M. ornithogaster</i>	1	1	2
>1+ budding <i>Candida</i>	0	5	8
> 95% of any single	0	2	2
>25% GNRs	5	23	37
Worm eggs	0	0	3
None of the above	74	34	154
Total	83	67	200 birds with 213 conditions (some multiple)

Table 7 shows that UL birds were more likely to show the following patterns compared parrots that were HN.

- > 25% GNRs concurrently with >1+ bacteria. Some normal cockatiels appeared to have higher levels of GNRs than other species.
- >1 budding *Candida* spp. per oil immersion field. Unhealthy Rainbow Lorikeets with high budding *Candida* spp were over represented compared with other species.

In addition

- While not statistically significant, one bird with > 95% Gram positive cocci and one with >95% Gram positive rods were UL.
- There was a trend between loose droppings and the presence of flagellates, worm eggs, coccidia and/or *M. ornithogaster* but many of these birds were apparently healthy at the time of testing and numbers were insufficient to draw statistically valid conclusions.

DISCUSSION

Gallinaceous birds and pigeons were not included in the study as they typically have higher levels of GNR in their normal intestinal flora (>25%), so Gram stains are less useful. Passerine birds typically have much lower levels of bacteria than psittacine birds. While Gram stains may be useful in passerine birds, because patterns appear to be different they were not included in this study.

Faecal wet preparation is a rapid screening test to identify worm eggs, coccidia and flagellates. A faecal Gram stain is useful to distinguish Gram positive from Gram negative bacteria and allows for easier and more accurate identification of budding *Candida* spp and *M. ornithogaster* than using a wet preparation alone. The tests are complementary, both are non-invasive, inexpensive to run, relatively easy to interpret, have rapid turn around times and can be carried out in house. The tests often provide quick and useful information as to what drug may be helpful in an individual case, particularly as both yeast and bacterial infections are commonly

associated with loose droppings and to simply treat such cases with empirical antibiotics could make either *Candida spp* or *M. ornithogaster* infection worse and could fail to treat other common infectious agents.

Some literature suggests that anything more than an occasional Gram negative rod or fungal organism is usually abnormal in parrot droppings (Fudge, 1996; 2010; Dahlhausen, 2010). This statement appeared to hold true for budding *Candida spp* in this survey but not with the presence of Gram negative rods, where 61% of the healthy birds had 5-25% of Gram negative bacteria. There only appeared to be a correlation between birds with over 25% Gram negative and clinical signs of disease.

Limitations of the tests need to be appreciated (Rosenthal, 2006). If the wet preparation sample is not fresh ('steaming') the frequency of identifying flagellates may be decreased. If the sample size is tiny the likelihood of seeing any organisms may be reduced. If the smear is too thick it can be difficult to see organisms, so it should be smeared more thinly. Thin smears may be over-decolourised with Gram positive organisms appearing Gram negative. Clinical judgment is needed in interpreting the tests (Isaza, 2000; Harris, 2009).

Absence of worm eggs, coccidia, flagellates or *M. ornithogaster* on wet preparation does not rule out the presence of these organisms in a bird but presence would generally prompt treatment. Gram stains do not identify the species of bacteria present, nor do they demonstrate that bacteria present are the cause of particular clinical signs shown in an individual bird. For example, *Trichomonas sp* was identified on crop wash on some budgies with high levels of Gram negative rods on faecal Gram stain. Abnormalities should prompt consideration of other diagnostic tests to search for other or underlying causes of disease.

REFERENCES

Dahlhausen RD. (2010). The avian Gram stain. *Small Animal and Exotics Proceedings of the North American Veterinary Conference*. Volume 20, Orlando, Florida, USA, 7-11 January, 2006. pp 1610-1613.

Fudge, A. (2010). <http://www.californiaavianlaboratory.com/micinfo.html>

Fudge, A. (1996). Avian Microbiology, chapt in *Disease of Cage and Aviary Birds*, 3rd ed, Roskopf, W and Woerpel, R (eds) Williams and Wilkins, Baltimore, pp 795-805.

Harris, D. (2009). Clinical Tests. *Handbook of Avian Medicine*, Tully, T., Dorrestein G and Jones, A (eds), Elsevier, Edinburgh, pp. 43-51.

Isaza, R. (2000). The Gram Stain in Avian Practice, Round Table Discussion, *Journal of Avian Medicine and Surgery*. 14: 194-198.

Rosenthal, K (2006). Microbiology: revisiting the Gram stain and culture. *Small Animal and Exotics Proceedings of the North American Veterinary Conference*. Volume 20, Orlando, Florida, USA, 7-11 January, 2006. pp 1575-1577.