WHAT YOU CAN SEE IN A PSITTACINE FECAL GRAM'S STAIN

In psittacines, the fecal Gram's stain is a significant part of a complete patient evaluation. Although not definitive in making a diagnosis, it provides a visual screen of the percentages of bacteria present in the gastrointestinal tract at the time the sample was collected and smeared. With the help of this information, the veterinarian can determine the next diagnostic step — whether to proceed to a culture and aggressive therapy, or to treat conservatively with husbandry changes.

Normal intestinal flora of parrots, seen as gram-positive (blue) bacteria on a fecal Gram's stain, represents both aerobic and anaerobic bacteria such as *Bacillus, Corynebacterium, Streptomyces, Lactobacillus, Streptococcus* and *Enterococcus* spp.[1] The anaerobic portion is difficult to grow using standard laboratory techniques, so the Gram's stain is the only practical means to assess the entire group.

Enterobacteriaceae are gram-negative (red) bacteria that include pathogenic (e.g., *Salmonella, E. coli, Acinetobacter*) and nonpathogenic species/strains. Enterobacteriaceae are not normal components of unstressed parrots' microflora.[1]

A Gram's stain is not intended to replace a culture for the evaluation of pathogenic bacteria in a sick bird. On the other hand, many healthy birds may show some transient Enterobacteriaceae on culture; therefore, cultures are not warranted in evaluating asymptomatic birds.

TECHNIQUE FOR PERFORMING A FECAL GRAM'S STAIN

1. With the wooden end of a cotton-tipped applicator, pick up a small amount of feces and apply it to a pre-cleaned glass slide. Use the applicator stick to spread the sample into a uniform, thin, even film, using a single swath.
2. Heat-fix the sample for 5-6 seconds by applying the flame from a cigarette lighter to the underside of the slide.
3. Prepare to stain by placing the slide on a staining tray.
   - Apply 3 drops gentian violet to the sample and allow to stand for 30 seconds. This stains all bacteria blue.
   - Rinse with water (elevate one end of the slide to drain excess water).
   - Apply 3 drops Gram's iodine and allow to stand for 30 seconds.
   - Rinse as above.
   - Apply 5 drops 95% ethyl alcohol to decolorize blue stain from gram-negative bacteria.
   - Rinse immediately, as above.
   - Add 5 drops saffron to stain gram-negative bacteria red.
   - Rinse immediately, as above.
   - Blot dry with lens paper or tissue.
4. Scan the slide under low microscopic power for an ideal evaluation site and apply 1 drop immersion oil to the site.
   - Using the oil immersion lens, scan several fields for a further idea of uniformity.
   - Choose a uniform field and begin to estimate the total number of bacteria. One way to do that is to count 10 bacteria, assess the proportion of the entire field occupied by those 10 bacteria, and then estimate the total bacterial population per 1000x field.
5. Record the results (see Table 1).

The entire process takes an experienced person less than 2-3 minutes to complete.

OBTAINING A FECAL SAMPLE

The most reliable results from fecal Gram's stains in the clinical setting are obtained with a consistent technique performed on fresh feces by a single person. Fecal samples obtained in the examination room are not optimal because of the nervousness of the patient. Ideally, the owner should be instructed to collect a sample at home and keep it cool until arrival at the office. This prevents proliferation of saprophytic gram-negative bacteria that may be interpreted as pathologic.

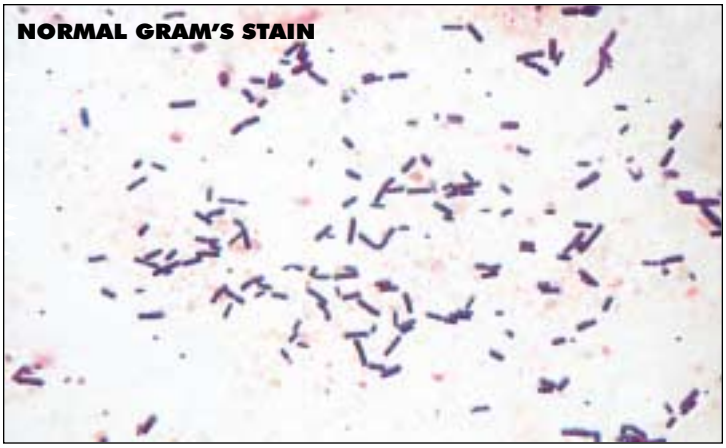

FIG 1 Budgerigar, 4-year-old male: Hx = Apparently healthy bird, fed Harrison's Bird Foods.™ CS = none. GS = Normal distribution of organisms: 157 total bacteria per field, 70% gram-positive rods, 30% gram-positive cocci, 0 gram-negative bacteria, 0 yeast. Digestion of food is complete.

COMMON CLINICAL GRAM'S STAIN RESULTS

The following images represent psittacine fecal Gram's stains commonly seen in clinical practice (1000x oil field). Most birds were fed primarily a seed-based diet plus some supplements. Distribution of fecal components and possible causes are described.

FIG 2 Cockatiel, 14-year-old male: Hx = Bird presented for boarding, seed diet. CS = Dull feather color, retained pin feathers. GS = 55 bacteria per field; 90% gram-positive rods, 10% gram-positive cocci. Hyperkeratotic cell with characteristic straight sides suggests intestinal microflora imbalance, probably due to malnutrition, early liver disease. Rx = Conservative.

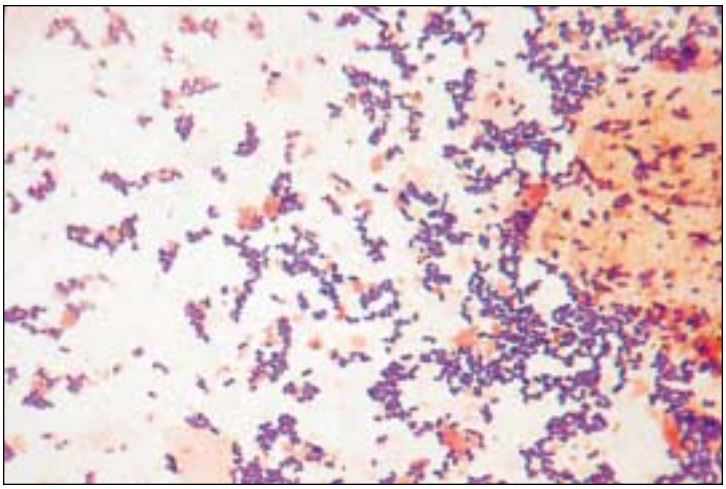

FIG 3 African grey parrot, 4 years old, sex unknown: Hx = Intermittent vomiting or loose stool, not as playful. GS = 400 bacteria per oil field, 95% gram-positive short rods, 5% gram-positive rods, 0 yeast. Overgrowth of intestinal bacteria, enterotoxemia, malnutrition. Rx = Aggressive.

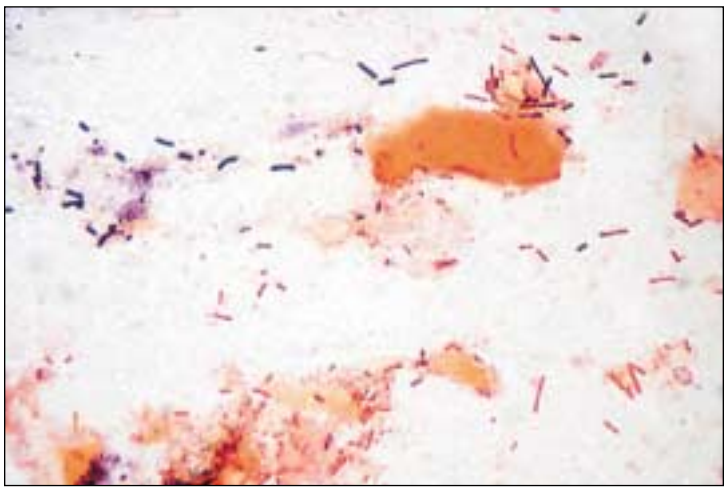

FIG 4 Psittacine: Iatrogenic gram-negative rods due to staining error. An error is suspected when the demarcation of gram-positive and -negative is linear and the groups are similar in shape and size, differing only in color. Note the presence of a normal intestinal epithelial cell, which is rounded and takes on a blue color. Compare this to the straight, pointed edges of the hyperkeratotic cell in Figure 2. Rx = None.

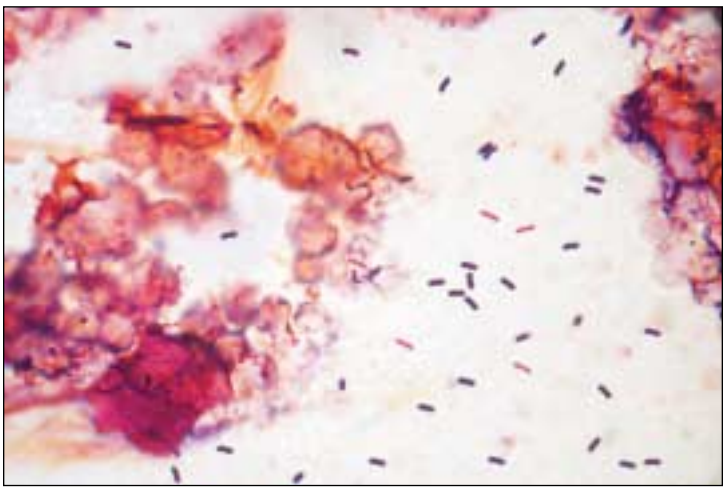

FIG 5 Amazon parrot, 8-year-old, female: Hx = Finicky eater, occasionally grumpy. CS = Failure to molt correctly, balding of feet, obvious layering of beak, overgrowth of nails, minor feather-picking. GS = 40 bacteria per field, 90% gram-positive rods, 0% gram-positive cocci, 10% gram-negative rods. (The normal binding of urates by protein is occasionally seen in fecal Gram's stains.) Rx = Conservative.

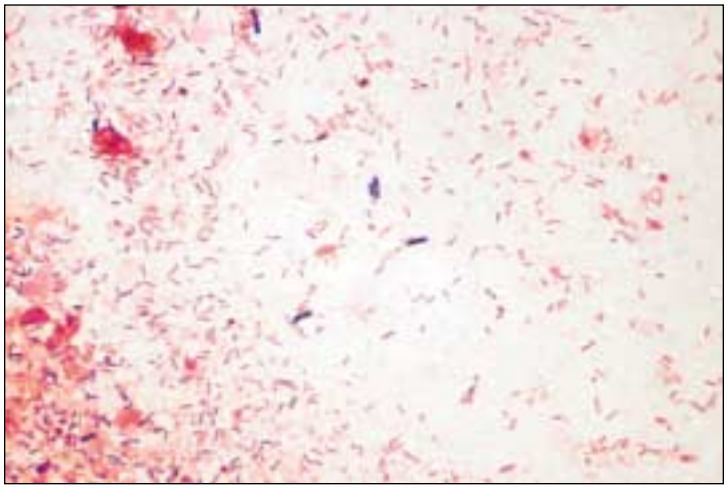

FIG 6 Severe macaw, 7 years old, sex unknown: Hx = Depressed, not eating, weak. CS = Underweight, scant feces, dark yellow urine and urates, malcolored feathers. GS = 200 bacteria per field, 1% gram-positive rods, 0% gram-positive cocci, 98% gram-negative rods. Rx = Aggressive.

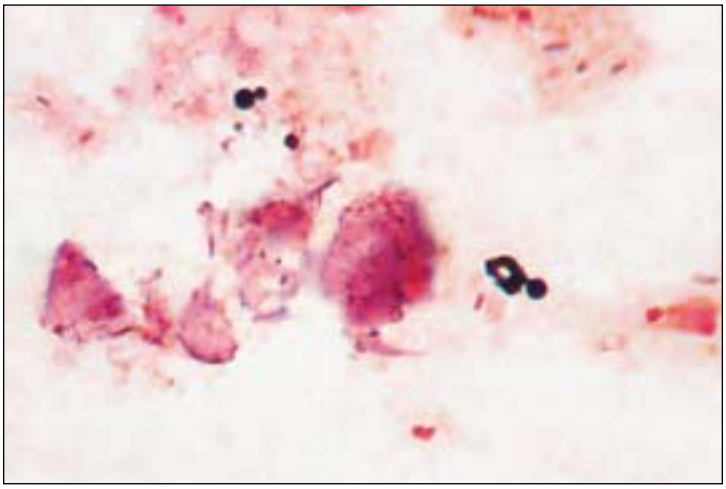

FIG 7 Meyer's parrot, 6 years old, sex unknown: Hx = Diet of seeds and supplements, treated previously for bacteria. CS = Depressed, fluffed, poor appetite. GS = Scant bacteria, two budding yeast organisms, suggesting early malnutrition. Rx = Aggressive.

FIG 8 Ring-necked parakeet, 9-year-old male: GS = Scant gram-positive bacteria, occasional gram-negative, many apparent bacterial forms and colors; invasive filament of yeast budding bi-directionally. Rx = Aggressive.

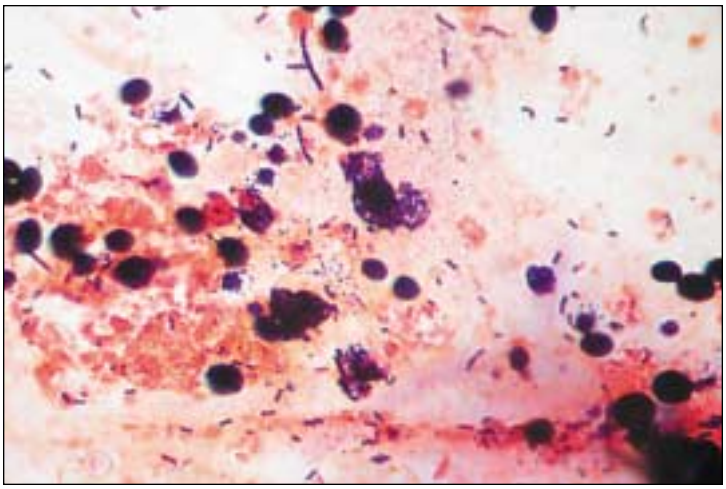

FIG 9 Cockatiel, 8-year-old female: GS = 80 bacteria per field, 80% gram-positive rods, 20% gram-positive cocci; 20 non-budding, yeast-like structures (possibly from bakery products in diet, not clinically significant). Rx = None.

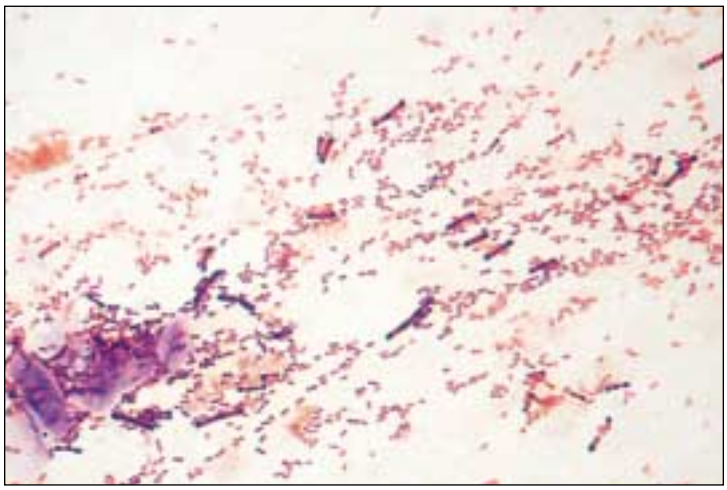

FIG 10 Umbrella cockatoo, 6-year-old female: Hx = Exposure to carnivorous pets, seed only diet. CS = Fetid stool, weight loss, passing undigested food. GS = 200 bacteria per field, 10% gram-positive rods, 90% gram-negative rods, of which 50% are *Clostridium* sp. Rx = Aggressive.

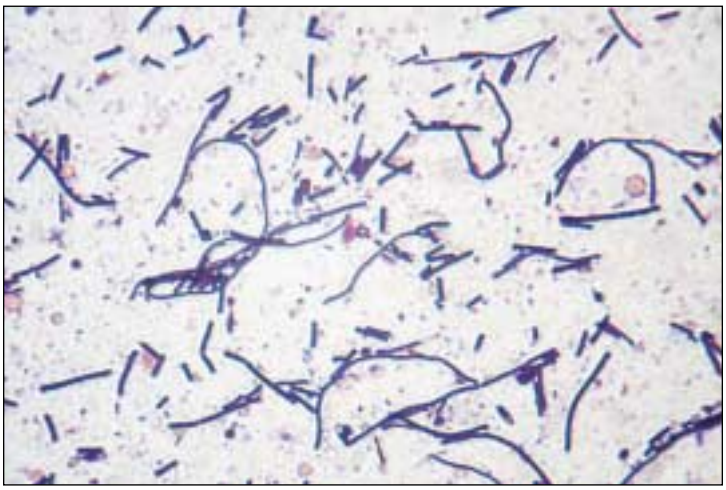

FIG 11 Budgerigar, 4-year-old male: CS = Digestive upset. GS = 200 bacteria per field, 5% gram-positive cocci, 95% gram-positive rods, of which half are large filamentous rods. Rx = Aggressive.

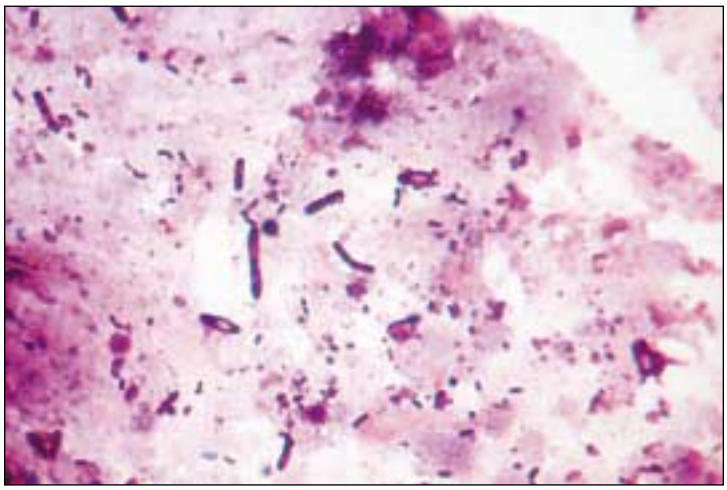

FIG 12 Moluccan cockatoo, 7-year-old male: CS = smelly stool. GS = 50 bacteria per field, 90% gram-positive rods, 10% gram-positive cocci, 30 *Clostridium* sp. organisms. Rx = Aggressive.

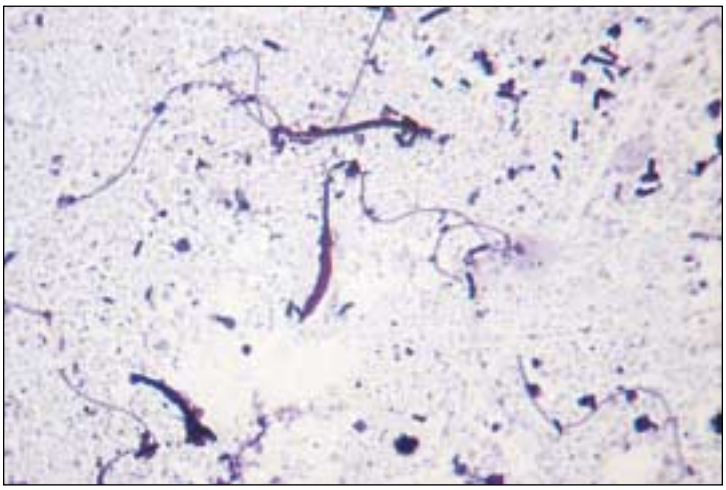

FIG 13 Budgerigar, 3-year-old male: Hx = Frequent masturbation. GS = Presence of sperm. Rx =None.

KEY Hx = History CS = Clinical Signs GS = Gram's stain results Rx = Therapy

FIG 14 Psittacine: Various forms of gastrointestinal diseases can be suspected if digestion of fiber or dietary ingredients is improper. Top = Normal fiber content of feces. Bottom = Undigested fiber.

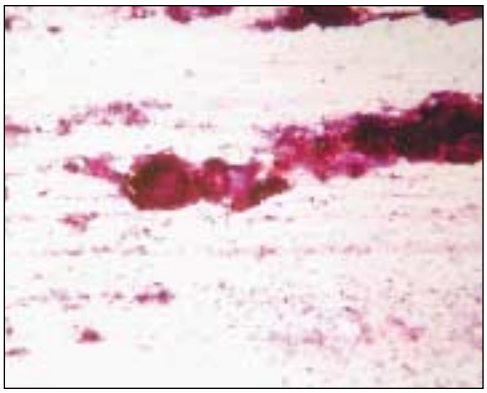

FIG 15 Psittacine: GS = Large amounts undigested fiber (low microscopic power).

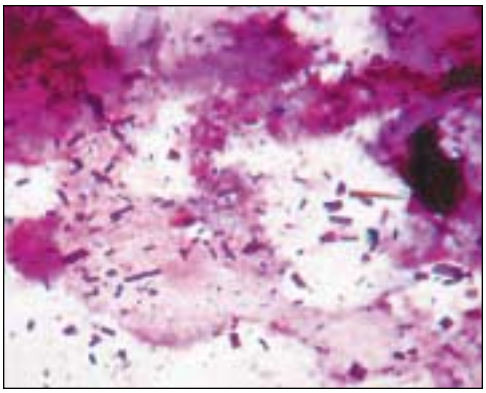

FIG 16 Psittacine: GS = 20 bacteria per field, 100% gram-positive rods, lots of undigested food particles cluttering field, suggesting some form of gastrointestinal disturbance.

INTERPRETATION OF FECAL GRAM'S STAIN RESULTS

According to Cheville,[2] "All body surfaces and orifices have characteristic normal resident microflora, the secretions of which prevent overgrowth of pathogenic bacteria — for example, intestine. These normal microbial populations reduce growth rates of some pathogenic bacteria through competition for nutrients and adhesion sites."

Normal intestinal secretions and the by-products from digestion are the most significant factors maintaining normal bacterial populations. Microflora in parrots degrade various fibrous carbohydrates (for energy), digest protein, assimilate nitrogen, and, in combination with cholesterol, produce more bacteria. This bacterial proliferation and fermentation produces acids and other protective by-products. Such acids inhibit the presence of gram-negative rods and yeast.

An imbalance in the homeostasis of the bird would result in upset of the normal functions of the microflora, and thus the distribution of bacteria in the gastrointestinal tract.

A healthy psittacine should have predominately gram-positive rods and cocci and less than 1% gram-negative rods.

Malnutrition and liver disease, which are very common in pet birds, are characterized by changes in the number and distribution of bacteria on the fecal Gram's stain. In the early stages, the change is reflected by:

- decrease in total bacteria
- decrease in percentage of gram-positive cocci.
- increase in gram-positive rods.

In the later stages of malnutrition and liver disease, the Gram's stain generally shows:

- increase in presence of gram-negative rods (the more gram-negative rods, the more pathologic the situation)
- presence of yeast (the more budding yeast per field, the greater the likelihood that the immune system is breaking down).

TABLE 1
Recording Results of Fecal Gram's Stain

Date _____

Species _____

Case ID _____

Results:

_____ total bacteria/1000x field
_____ % G+ rods/field
_____ % G+ cocci/field
_____ % G- rods/field
_____ number yeast/field
_____ % budding yeast
_____ high fiber in feces
_____ undigested food
_____ parasites
_____ clostridia organisms
_____ hyperkeratotic cells
_____ normal intestinal cells

REFERENCES

1. Ritchie BW, Harrison GJ, Harrison LR (eds): Avian Medicine: Principles and Application. Lake Worth, Wingers Publishing, 1994, pp 951, 968, 978.

2. Cheville NF: Introduction to Veterinary Pathology 2nd ed. Ames, Iowa State University Press, 1999, p 155.

3. Ritchie BW: Case report presentation. Proc No Am Vet Conf, 2000.

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