Basic Avian Diagnostics

Patricia Macwhirter*

Introduction

Commercial laboratories in Australia currently offer only limited diagnostic testing for birds so diagnostic work is generally carried out in house in most avian practices. These are routine procedures that are widely used in avian practice.

Cost is often a greater concern in avian practice than it is with other companion animals. Owners should always be offered what the veterinarian considers to be optimal care in terms of diagnostic testing and treatment procedures, but it will often be the owners wish that recommended procedures be modified because of financial constraints.

Be ready to prioritise tests in terms of cost and degree of invasiveness compared with likelihood of providing a diagnosis that will result in the condition being resolved. A careful history, physical examination and knowledge of common clinical problems in individual species is needed to do this effectively.

Bacteriology

Gram staining

Useful to identify the presence of bacteria, megabacteria and yeast but gives no information as to antibiotic sensitivity and there are often marked differences in what is seen on initial Gram stain and what grows in aerobic culture.

Choanal, cloacal and/or fresh faecal gram stains are routinely used to assess bacterial flora in cage birds. In general there should be a mixed Gram-positive flora with less than 5% Gram-negative organisms.

Bacteria can be isolated in low numbers from the liver and kidney of normal birds because of their portal circulation and lack of filtering lymph nodes.

Isolating any organism in large numbers in almost pure culture suggests that the organism may be part of the disease process.

Culture and Sensitivity

Resistant bacterial infections are common in birds and often occur in conjunction with psittacosis. Appropriately interpreted, bacterial culture and sensitivity give specific information about drugs that are likely to be useful in treatment.

* **Highbury Veterinary Clinic, 128 Highbury Road, Burwood, Victoria, 3125**

We find it beneficial to do in-house bacteriology as we can encourage the owner to have a Gram stain and plate (split blood/McConkey agar) done and the additional cost of identification and sensitivity testing is only incurred if abnormal findings occur. Most owners will accept this while many will resist a full charge from the outset for ID, culture and sensitivity when the likelihood of finding the cause of the problem may not be very high.

Don't just set up sensitivity plates without knowing the organism with which you are dealing. It will take time and study to upgrade your bacteriology skills to be able to key out organisms, but doing this will help in your general understanding of disease processes in birds.

Recognise your limitations and be ready to send difficult cases to outside laboratories

Gram-negative bacteria

Normal flora - *Enterobacteriaceae* are part of the normal flora in gallinaceous birds and pigeons. They should only occur at low levels (<5% of bacteria present in cloacal Gram stains) in psittacine birds. They are not normally found in finches.

Enterobacteriaceae

Escherichia coli - disease syndromes

- Colisepticaemia: Serofibrinous inflammation of many body organs.
  Sick bird signs, polyuria, diarrhoea, death
- Coligranulomas - esp in gallinaceous birds
- Rhinitis, rarely pneumonia
- Genital tract infections - esp in canary hens, egg related peritonitis
- Joint infections
- Localised enteritis

Salmonella spp

- Egg transmitted, often a problem in pigeon lofts
- L forms can make both culture and treatment difficult, try clindamycin
- Vaccine is currently being trialed
- Clinical syndromes include: Acute bacteraemia
  Neurological signs
  Joint infections
  Ocular lesions

Yersinia spp

- Associated with rodents
- Typically see sick bird signs, diarrhoea and acute deaths in aviaries
- On autopsy microabscesses in the liver and spleen are typical.
- Common problem in finch aviaries, also seen in psittacines.

Pseudomonas & *Aeromonas* spp

- Frequently found in aquatic environments, clean up the water supply if these bacteria re identified
- Causes septicaemia, diarrhoea, dyspnoea & death

Klebsiella spp

- May be opportunists or primary pathogens
- Associated with GI, renal and lung disease

Campylobacter spp

- Enteritis & liver disease
- Dogs may be a reservoir for human infections with Campylobacter, suggest keep dogs away from aviary

Pasturella spp

- Characteristic bipolar staining
- Cause respiratory disease, acute septicaemia and death
- Infected rodents and free ranging birds are important reservoirs
- Associated with cat bite wounds
- Epornitics are common in migrating waterfowl in the northern hemisphere
- *P. multocida* (which causes fowl cholera) and *P. gallinarum* are the most common species

Haemophilus spp

- Causes coryza in chickens, associated with conjunctivitis, sinusitis and rhinitis in psittacine birds & pigeons. Special culture requirements.

Gram-positive bacteria

Staphylococcus spp

- May be part of the normal flora or a primary or secondary pathogen
- L forms and rapid development of resistance to disinfectants can make treatment and control difficult
- Associated with
    - Acute septicaemia
    - Arthritis
    - Osteomyelitis
    - Vesicular dermatitis
    - Omphalitis
    - Pododermatitis
    - Thrombi formation in arterioles and capillaries can lead to ischaemic necrosis of extremities
    - Delayed hypersensitivity reaction may complicate recovery

Streptococcus spp and *Enterococcus* spp

- Part of the autochthonous flora of the skin & mucosal surfaces but also associated with disease.
- Natural infections occur by the oral route and are most common in postnatal and growing birds
- Disease syndromes include:
- Vertical transmission can cause embryonic deaths, omphalitis & post hatching development problems
- Septicaemia with embolic-thrombotic systemic disease
- Arthritis
- Endocarditis
- Pneumonia, tracheitis
- Peritonitis, salpingitis, oophoritis

Mycobacterium spp

- Affects all bird species, most species form granulomas but not in psittacines.
- Causes chronic weight loss, diarrhoea and death. Most affected birds show a marked heterophilia. Radiographs may indicate granulomas (rare in Australia) which must be differentiated from fungal granulomas
- Bacteria is highly resistant to environmental extremes and can survive for long periods. Some northern hemisphere zoos have chosen to depopulate their collections because of persistent problems with avian tuberculosis. Migratory waterfowl can cause recurrent out breaks (eg Slimbridge in the U.K.)
- See numerous acid fast rods on impression smears of tissues, can also pick up bacteria in faeces but only intermittently. Culture can be difficult
- Occurs but is not widespread in Australia. Usually diagnosed on autopsy.
- Treatment using human anti-tuberculosis drugs has been successful in some cases but is difficult and not recommended because of the human health risk.

Erysipelothrix rhusiopathiae

- Causes diarrhoea, dermatitis, arthritis and peracute deaths.
- Most common in waterfowl and turkeys but can occur in other species.
- Rodents, pigs and raw fish have been implicated as reservoirs for the bacterium.
- Zoonotic potential

Listeria spp

- Causes sporadic deaths, pericarditis and CNS signs
- Canaries more susceptible than other birds
- Latent infections occur.

Clostridium spp

- Part of the autochthonous flora in raptors and birds with well developed caeca such as *Anseriformes* and gallinaceous birds
- The genus produces potent toxins, various Clostridial infections can produce

similar-appearing diseases

- Necrotic or ulcerative enteritis
  - Reported in game birds and lorikeets, usually young birds, adults are more resistant.
  - Acute enteritis and death
  - *Clostridium perfringens* is the aetiological agent
- Gangrenous dermatitis
  - Caused by *Cl perfringens*, *Cl novyi* or *Cl septicum*
  - Organisms colonise damaged skin
  - Regional feather loss with red/blue/black skin discolouration, gas accumulation, toxaemia and death
- Botulism (Limberneck)
  - Caused by ingestion of Cl. botulinum neurotoxins.
  - Found in decaying meat or vegetation. Maggots that feed on decaying material are resistant to the toxins and may serve as a source of intoxication for susceptible species.
  - Free-ranging waterfowl are particularly susceptible following periods of drought or flooding.
  - Toxins enter the body through the intestinal wall, enter the ends of nerves and irreversibly bind to the neuromuscular junctions and block the production of acetylcholine.
  - Toxins also damage vascular endothelium causing oedema and petechiation.
  - Clinical signs include flaccid paralysis, diarrhoea & (sometimes) feather loss. Death is due to respiratory paralysis.
- Tetanus - Birds are resistant

Other Gram positive bacteria

- *Bacillus* spp., *Corynebacterium* spp., *Streptomyces* spp. and *Lactobacillus* spp. are frequently recovered from normal birds and are generally not considered to be pathogenic.

Megabacteria

- Very large (1 x 90 µm) Gram-variable rod with some characteristics of fungi although it is a bacterium.
- Difficult to culture. Needs to be differentiated from Lactobacillus sp which looks morphologically similar but is easy to culture.
- Resistant to most antibiotics but responds to amphotericin
- Associated with progressive weight loss in English budgerigars. See ulceration and proventriculitis with numerous megabacteria present at the proventriculus / ventriculus junction.
- Also seen over a range of other species including wild caught galahs and cockatoos, finches, small parrots and ostriches. Low numbers may not be clinically significant.

Mycoplasma spp

- Well documented in poultry, significance in companion birds is less clear. often associated with multifactorial disease problems.
- Many strains have been isolated that are not associated with disease
- Culture requires specialised facilities so is rarely done but may be associated with sinusitis,

respiratory and genital problems and possibly joint disease.
- If suspected treat with tylosin or tetracyclines

Mycoses

- Often influenced by stress factors such as age (eg *Candida* in young birds), nutrition (eg aspergillosis in vitamin A deficient birds) or concurrent antibiotic therapy

    - *Candida albicans*
        - Opportunistic yeast that causes a variety of GI problems
        - May also see vent infections, skin lesions and respiratory infections in immunosuppressed adults
        - May be part of the normal flora if present in low numbers
        - Need to differentiate dietary yeast from pathogenic yeast. Yeast from dietary sources such as bread will not show budding forms ("Mickey Mouse ears") or hyphae and there should be a history of the bird eating a yeast source.

    Diagnosis

    - Identifying budding forms, hyphae and/or large numbers of organisms in association with clinical signs. Use Gram staining or Diff Quik on crop wash fluid.
    - May not always find the organism because it may be deep in the mucosal lining
    - Typical Turkish Towel appearance to affected crop lining
    - Look for another primary cause for the disease!

Aspergillus spp

- Associated with brooder pneumonia in poultry and several respiratory syndromes in other avian species. Penguins are particularly susceptible.
- Mouldy organic material, particularly straw, is a common source of spores.
- Predisposing factors -Vitamin A deficiency causing squamous metaplasia and a nidus for infection in the respiratory passages, long term antibiotic or corticosteroid therapy, damp, poorly ventilated housing.

    - **Syndromes**
        - "Bread mould" (hyphal form) air sac and respiratory lesions
            - Respiratory distress, sick bird signs, weight loss
            - Lesions may be visible radiographically
            - May be able to culture organism from trachea (beware! can be part of normal flora) or endoscopically from air sacs.
            - Gross lesions often have characteristic appearance
        - Generalised granuloma formation
            - Clinical signs as for hyphal form
            - Need to differentiate this form from pyogenic infections. Check histopathology or impression smears for characteristic "medusa head" hyphal formations
            - Syringeal granuloma which may cause respiratory obstruction
            - Ocular aspergillosis seen in chicks as a white exudate within the conjunctival sac.

- **Diagnosis**
  - Radiology, haematology (marked heterophilia)
  - Identification of typical lesions on endoscopy
  - Culture, histopathology showing organism in association with clinical disease.

Cryptococcus neoformans

- Saprophytic yeast, isolated frequently from bird droppings. Two forms:
  var gati: associated with River Red Gum trees;
  neoformans: is not so associated.
- Uncommon cause of sinusitis and CNS signs. Gelatinous material is produced.
- Characteristic encapsulated yeast like organism stains with India ink
- Zoonotic potential

Histoplasma capsulatum

- Similar to cryptococcus but less common.

Trichophyton gallinae

- Dermatophyte associated with dermatological problems in a variety of species, eg Fowl Favus
- Culture on Sabaroud's agar
- Zoonotic potential

Systemic mycosis

- Associated with a variety of fungal pathogens including *Rhizopus, Mucor, Trichosporon* and *Penicillium* spp.
- Diagnosed by histopathology & culture.

Parasitology - faecal smears and flotation

- Avian faecal flotations are carried out as for any other species. Be particularly careful to check under x 40 for coccidia and to train staff to do this as coccidia are common in birds and can be overlooked if staff are used to dealing only with dogs and cats.
- Staff should also be trained to recognise pollen grains and other plant material as these can be confused with coccidia.
- Some practitioners feel that there is little difference in doing direct smears compared with faecal flotations.
- Direct faecal wet preparations are also needed to identify flagellates such as *Hexamita* (often in ducks), *Giardia* (rare in Australia), *Cochlosoma* and other "E.R.P.S." (Emtryl responsive protozoans, species not identified).

Crop wash

- Aspirate fluid, examine a wet preparation for flagellates, air dry and Gram stain for bacteria & fungi. It is difficult to identify protozoa on stained preparations but it is easy to see them moving in wet preparations

Haematology and blood biochemistry

- Campbell(1) or Ritchie, Harrison and Harrison (2) are the best sources of colour photographs of blood cells and cytology examples to help with cell identification in an isolated practice situation.
- Avian practitioners vary as to whether they choose to go for blood work, radiology or microbiological testing for birds that come in with non-specific sick bird signs. In our practice, depending on the individual case, we tend to go for microbiological testing first as this will give specific information about appropriate drugs for treatment. Radiology is often our second choice because heavy metal toxicity is such a common diagnosis and radiographs give a great deal of other information. Haematology and biochemistry are often our third options but they can sometimes give valuable information that can not be found by other means.

Blood collection

- For most cage birds the jugular venipuncture is the simplest and most effective way to collect a blood sample. It can be done single handed, and the volume of blood that can be easily collected is sufficient. Cutaneous ulnar, medial metatarsal or toe nail clip are generally less satisfactory alternatives but they are used by some practitioners.
- We use a heparinised tuberculin syringe with a 25g or 27g needle for collection.
- Maximum amount that should be collected is 1% of the bird's body weight (0.35 ml for an average 35g budgie) but take the minimal amount necessary to run the tests required. 0.2 ml will allow enough for a total white cell count and differential, PCV and total solids and several biochemistries (we usually run AST, uric acid and glucose using a Reflotron).

PCV and total solids

- These are run as for mammalian species using a microhaematocrit centrifuge and a refractometer. Interpretation is generally as for mammalian species. Some birds may have very low total solid and still survive.

Haematology

- Cover slip smears, are the most widely used method to give an even spread of red and white cells for white cell counts and differentials.
- Cells can be stained with Diff Quick or a variety of other haematological stains (see texts)
- Estimated total white cell counts are done by taking the average number of white cells per high powered field and multiplying the number by 2000. This figure is based on having a single monolayer of touching cells. If the spread of cells is too thin it may read low, whereas with thick layers it may read too high. Counts may also be done using Unopettes designed for eosinophils.
- Differentials are done as for mammalian species. See the recommended texts for cell identification. These will also be shown as slides in the lecture.
- Interpretation - see summary tables in recommended texts.

Bone marrow biopsy can be done using a disposable spinal needle accessing the proximal tibiotarsus.

Biochemistry

- Overseas comprehensive panels are available from external laboratories specialising in avian and exotic species. These can be done on very small sized blood samples. This service is not being offered in Australia, panels generally available here are much more limited in scope. Some useful tests include:

    - **Aspartate aminotransferase - AST (GOT).** Usually indicative of liver or muscle damage, more specific than ALT or alkaline phosphatase and generally run in preference to them. Levels> 230 U/l are considered abnormal.

    - **Creatinine kinase**
        - Useful to run in conjunction with AST to differential liver damage from muscle damage. CK is elevated in muscle damage, for example with Vitamin E deficiency or irritant injections.

    - **GGT, GLDH, LDH** are non specific and seldom used in avian diagnosis.

    - **Amylase** may be elevated with damage to the pancreas, liver or small intestine. Increased enzymic activity can be seen with pancreatitis. Normal reference values for most species are <600 U/l. Lipase values may also be elevated in pancreatitis but few studies have been done on the diagnostic significance of the enzyme in birds.
    - **Bile acids.**
        - If liver function is impaired, bile acids are not resorbed from the blood and consequently the proportion of excreted bile acids remaining in the blood increases. It is a fairly specific indicator of liver malfunction but the test is not readily available to avian practitioners in Australia.
    - **Calcium** concentrations may rise with hypervitaminosis D, bone tumours or dehydration. It may fall in hypocalcaemia syndrome of African Grey Parrots (a unique form of hypoparathyroidism where the calcium is not released normally from the bone).
    - **Cholesterol** is non-specific and of limited diagnostic value. Very high levels usually accompany lipaemia
    - **Creatinine**. Birds excrete creatine in urine before it is converted to creatinine, consequently this test is not as useful as in mammals. It may be elevated with renal damage or egg-related peritonitis but is rarely run.
    - **Glucose**
        - In birds glucagon rather than insulin is the primary regulator of glucose metabolism.
        - Heparinised tubes are okay to use for glucose estimations as avian red blood cells depend primarily on fatty acid metabolism and use very little if any glucose.
        - Elevations occur with diabetes mellitus, "deranged glucose metabolism" syndrome and egg-related peritonitis. Decreases may occur due to hepatic dysfunction, starvation, increased utilisation due to septicaemia, convulsions, neoplasia.

    - **Phosphorus, iron and total iron binding capacity** have not proven to be a great diagnostic value.

    - **Uric acid**
        - Elevated levels are generally associated with renal disease, normal levels do not guarantee that the kidneys are healthy.
        - If a toe nail clip is used for collecting blood uric acid levels may be elevated because of urate contamination on feet.

- **Delta-aminolevulinic acid dehydrogenase**
  - See decreased activity with heavy metal toxicity

Cytology

Same general principles apply as for mammalian species. Check the recommended texts for identification of avian cells. Stains used include Diff Quik for general cytology,
Macchiavello for suspected *Chlamydia*, acid-fast staining for suspected mycobacteria and Gram staining for yeast or bacteria.

- **Swabs**
  - Choanal and cloacal swabs are routinely set up for culture and Gram stain. Swabs can also be taken from inflamed conjunctiva (check for *Chlamydia* & bacteria), abnormal lesions elsewhere on the body, ulcers etc.
- **Joints**
  - Useful to differentiate gout from infection. Gout will appear as needle-shaped uric acid crystals. Heterophils are generally seen with joint infections.
- **Abdominocentesis**
  - DD neoplasia, infection, egg-related peritonitis, peritoneal cysts, haemoperitoneum, ascites.
  - General principles as for mammalian species.

    Check general appearance:
    - clear (peritoneal cyst),
    - blood (bleeding neoplasm or trauma)
    - yolk-like (egg-related peritonitis)
    - purulent (infection)
    - serosanguinous (ascites, neoplasia) **Do a S.G. to determine whether fluid is a:**
    - transudate (SG <1.020, peritoneal cyst, ascites due to cardiac insufficiency, hepatic disease, hypoproteinemia)
    - modified transudate (SG <1.020 but cellularity 1,000-5,000/ul: long standing transudate, mesothelial irritation)
    - exudate (SG >1.020, inflammation, neoplasia, egg-related peritonitis, haemorrhage)

    Prepare a cytological sample of the fluid or sediment and stain with Diff Quik or other cytological stain. Look for evidence of:
    - Inflammation
      - heterophilic - sepsis, severe irritation
      - macrophagic - low grade chronic irritation, foreign body
      - mixed - intermediate
    - Non inflammatory
      - Fat Globules (Yolk Material In Egg Related Peritonitis)
      - Neoplasia (mitotic figures, sheets of similar cells)
      - Urates
      - Acellular (transudates, peritoneal cysts)

Infra orbital sinus aspirate
Enter via the fleshy skin at the commisure of the mouth and direct the needle midway between the eye and external nares or pick a point of maximum extension of the sinus.
Prepare a sample for culture and stain with Gram Stain, Diff Quick, India Ink (for *Cryptococcus*) or acid-fast stain depending on index of suspicion.

Lump aspirates
May be useful to differentiate lipomas (see fat globules) from abscesses (see degenerating heterophils and macrophages) or resolving haematomas or neoplasia.

Contact smears
May be useful for ulcerative lesions in vivo but most commonly used in post mortem examination, for example:

- Serial wet preps and/or Gram stains may be done along the GIT to assess yeasts and bacterial flora.
- Impression smears of the liver and/or spleen are stained with Macchiavello to check for *Chlamydia* or Diff Quik to check for *Atoxoplasma* or Gram stained for bacteria or acid-fast staining done for mycobacteria. Impression smears of abnormal lesions may also be stained depending on suspected organisms or to check cytology.

***Chlamydia* testing**

- Definitive diagnosis requires identifying elementary bodies on impression smears of the spleen or liver. These stain distinctively pink with Macchiavello stain.
- Reticulate bodies may stain blue.
- Serology is widely used overseas but is not routinely available in Australia
- *Chlamydia* culture can be difficult, a negative culture does not rule out a diagnosis of chlamydiosis
- Birds with psittacosis generally have elevated WBCs and liver enzymes
- **Antigen capture testing**. There are several kits available, all of which may give false positive reactions due to interaction with other bacteria or false negatives due to intermittent shedding of the organism.
    - These kits have been developed for detection of *C. trachomatis* in vaginal samples in women but can be used for *Chlamydia psittaci* because both organisms share the same group-specific antigens.
    - Very high levels of *Staphylococcus aureus*, *Actinobacillus*, *Pasturella* or *E. coli* can cause false positive reactions on the test. Birds which may have coliforms as part of their normal gut flora (eg pigeons) are more likely to give false positive reactions. SureCell is less likely to give false positive reactions than other kits.
        - Clearview *Chlamydia* test (distributed by Vetafarm) is the cheapest and most widely used. The Kodak SureCell test gives fewer false positives but is more expensive to run.
        - These tests are useful if considered in conjunction with other evidence for psittacosis. They are not recommended as routine screening tests because of the problem with false positive and false negative reactions.

Urinanalysis

In birds with polyuria it is relatively easy to collect a urine sample if droppings are collected on aluminium foil. Urine specific gravity is tested with a refractometer and urine dipstix used to test biochemical parameters

- **SG** should range from 1.005 to 1.020. Water deprivation testing can be done as in mammals to test for diabetes insipidus/medullary washout syndrome vs psychogenic water drinking. Watch weight and do not allow the birds weight to drop more than 10% when doing the test. Diagnosing diabetes insipidus definitively is difficult because avian ADH is not readily available.
- **Protein** - there should be a trace in normal urine.

  - Many renal disorders will result in mild to moderate proteinuria
  - Non-renal causes of proteinuria include haematuria, haemoglobinuria and hyperproteinaemia due to increased production of immunoglobulins.

- **Glucose** - normally this is negative to trace
  - Elevations may occur with diabetes mellitus, egg-related peritonitis and deranged glucose metabolism syndrome
- **Ketones** - should be absent
  - Elevations occur with any shift in energy production from carbohydrates to fats. Catabolic processes such as severe hepatitis in combination with low blood glucose or diabetes mellitus can cause ketonuria.
- **Bilirubin** - should be absent
  - Biliverdin, the major bile pigment in birds should not react with the bilirubin reagent.
- **Blood** - should be negative or trace
  - May get elevations with haemoglobinuria associated with heavy metal toxicity or with haematuria associated with severe renal or urinary tract bleeding.
  - Faecal contamination can cause false positives in some cases
- **Sediment**
  - Casts may be seen with severe renal disease
  - Presence of any epithelial cells should be considered abnormal
  - More than 6 red and/or white blood cells per high power field is considered abnormal.
  - Bacteria are difficult to assess because of contamination with the faecal component of the droppings.

References

1. Campbell, T: Avian Hematology and Cytology. Iowa State University Press, Ames Iowa, 1988

2. Ritchie, B, Harrison, G, Harrison, L: Avian Medicine; Principles and Applications. Wingers Publishing Inc., Lake Worth, Florida, 1994