WHY DO BIRDS GET SICK?

Bob Doneley
West Toowoomba Veterinary Surgery
194 West St., Toowoomba
Queensland 4350

INTRODUCTION

The relatively close confines of captivity mean an increased pathogen load in the environment in which companion and aviary birds live. This increased pathogen load leads to greater exposure of these birds to viruses, bacteria, fungi, parasites and other pathogens, and consequently a greater risk of infection and disease.

> *"If I could live my life over again, I would devote it to proving that germs seek their natural habitat - diseased tissues - rather than causing disease."* Rudolf Virchow, 1821-1902

Rudolf Virchow, the German doctor, anthropologist, public health activist, pathologist, prehistorian, biologist and politician, is often described as the "Father of Pathology" and the originator of the theory of biogenesis the concept that living cells can arise only from pre-existing living cells. He argued that pathogens (germs) do not cause disease but, rather that they invade already diseased tissue. This went against the work of Louis Pasteur (1822-1895), who believed that bacteria and other pathogens were the primary cause of disease. Today, we acknowledge that both men were correct. While some pathogens are primary agents, capable of causing disease in their own right, many are secondary infections taking advantage of diseased tissue or a compromised immune system. Other bacteria, viruses and fungi sometimes found in birds are simply transient or even normal flora. Clinicians should not be content with diagnosing an infection in their patient based only on PCR, a culture or Gram stain the patient's history, clinical signs, and other ancillary diagnostics such as haematology should be used to confirm that the isolate is, in fact, significant. They must also always ask themselves, "Why does this bird have an infection?"

This paper discusses some of the risk factors associated with infectious diseases in companion and aviary birds (particularly parrots).

NORMAL FLORA AND FAUNA IN BIRDS

Normal Bacterial Flora

It is a long-held belief in avian medicine that the normal bacterial flora of healthy parrots is predominantly Gram positive bacilli; conversely, Gram negative bacteria have been considered abnormal and potentially pathogenic. (Fiennes, 1957; Fiennes, 1969; Fudge, 2001; Harrison and McDonald, 2006) Various surveys have been conducted of both healthy and diseased parrots to ascertain normal and abnormal bacterial flora (Jones and Nisbet, 1980; Bangert et al., 1988; Flammer and Drewes, 1988; Harrison and McDonald, 2006) and all agree that Gram positive bacilli are the predominant flora, but there is some disagreement on the significance and normality of the presence

of Gram negative bacteria.

Bangert et al. (1988) reported that faecal isolates from healthy parrots included Gram positive bacilli (*Lactobacillus* spp, *Bacillus* spp, *Corynebacterium* spp, *Streptomyces* spp), Gram-positive cocci, (*Staphylococcus epidermidis, Streptococcus* spp., *Aerococcus* spp., and *Micrococcus* spp) and, in a low number of birds, Gram negative bacteria (*E. coli*). They further reported that the number of birds yielding *Corynebacterium* spp. and gram-negative bacteria increased with age, whereas the number of birds yielding lactobacilli decreased with age. Flammer and Drewes (1988) reported that 91% of 506 clinically normal parrots had Gram positive bacilli recovered from cloacal cultures. However, *E. coli* was recovered from 31% of these birds, *Enterobacter* spp from 4%, *Klebsiella* spp from 0.6% and *Pseudomonas* spp from 0.8%. Species differences were noted: *E. coli* was recovered from 60% of the cockatoos (*Cacatua* spp) cultured (168 birds), but from only 18% of non-cockatoo species (338 birds). All birds were housed in the same facility with similar diets and husbandry, suggesting that these were species-related differences, rather than differences in diet and management. Jones and Nisbet (1980) cultured *E. coli* from the intestinal tract of 46% of 54 birds necropsied for non- intestinal diseases. These birds were all specimens from a zoological collection and could be expected to have had similar diets and husbandry.

On the other hand, Harrison and McDonald (2006) state that the normal faecal flora of parrots is comprised of 100% gram-positive, non-spore forming rods and cocci, and that Gram negative bacteria should not be present in the faeces of parrots on a healthy diet. This belief is reinforced by the work of Stanford (2003) that showed that Gram negative bacteria in the faeces of Grey birds (*Psittacus erithacus*) were reduced to almost zero after conversion from a typical seed-based diet to a nutritionally balanced one.

Respiratory tract flora appears to follow a somewhat different pattern. Fudge (2001) states that tracheal washes are usually sterile, but that contamination from the oropharyngeal area during collection of samples can be misleading. Drewes and Flammer (1988) and Tully (1995) state that the bacterial flora from the upper respiratory tract is predominantly Gram positive bacilli. Jesus and Correia (1998), on the other hand, cultured the choana of nineteen healthy birds; of the 36 bacterial isolates recovered, 26% were Gram positive bacteria and 62% were gram-negative bacteria. Isolates recovered were α-haemolytic *Streptococcus* spp, non-haemolytic *Streptococcus* spp, *Gemella morbillorum*, *Leuconostoc* spp, *Staphylococcus* spp, *Staphylococcus hominis*, *Bacillus* spp, *Actinomyces* spp, *Alcaligenes* spp, *Acinetobacter baumanii*, *Enterobacter cloacae*, *Erwinia nigrifluens*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Moraxella lacunata*, *Pasteurella* spp, *Pseudomonas alcaligenes*, *Pseudomonas stutzeri*, and *Xanthomonas maltophila*. Some of these bacteria are known as potential pathogens, suggesting that the resident flora of the upper respiratory tract of healthy birds can, given the right opportunity, behave as opportunistic pathogens.

In recent years attention has been given to culture-independent' assessment of normal bacterial flora in both mammals and birds. This work has identified that the majority of microbial species present in biological samples escape identification by use of standard culture techniques alone. In contrast, studies using molecular fingerprinting methods and/or sequencing of cloned microbial 16S rRNA (ribosomal DNA, rDNA) have been successful in identifying a greater number of bacterial species than standard culture techniques (Gray et al., 2007). In a limited study by Gray et al. (2007), 11 different bacterial species (from the genera *Staphylococcus, Pantoea, Escherichia, Lactobacillus, Lactococcus, Enterococcus, Curtobacterium, Stenotrophomonas, and Peptoniphilus*) were identified from wild and captive parrots, with a high variation in the microfloral composition among individual birds, and

between wild and captive parrots. *E. coli* was identified in 2 captive macaws, but not in wild macaws.

The findings discussed above suggest that the normal bacterial flora in healthy parrots, while predominantly Gram positive bacteria, may include some Gram negative bacteria as well. There appears to be variation between individuals and species, and between captive and wild birds. From a clinician's point of view it must be remembered that most patients presented for veterinary examination are rarely on a nutritionally balanced diet, nor is their husbandry identical or even optimal. As such, it can be expected that the bacterial flora in these birds will possibly include low numbers of Gram negative bacteria. This does not constitute evidence of a bacterial infection, nor does it always warrant antimicrobial therapy.

Normal Fungal Flora

Bangert et al. (1988) reported that in a survey of 61 clinically healthy parrots, *Candida albicans*, *Cryptococcus laurentii*, and *Aspergillus* spp. were isolated from 13 faecal cultures. *C. albicans* was isolated exclusively from 5 pet shop birds.

C. albicans is not regarded as a primary pathogen, and is rarely present in large numbers in the intestinal tract of healthy birds. However, non-budding yeasts are not uncommon, and may indicate a dietary source (e.g. bread, biscuits, and some lorikeet diets) rather than an infection. If budding yeast are seen, indicating “live” yeast, infection is more likely; if fungal hyphae are seen, the spectre of possible tissue invasion is raised.

Cryptococcus spp and *Aspergillus* spp are ubiquitous environmental organisms and, as such, their detection in a bird will require the clinician to make a distinction between infection and transient populations.

Normal Viral Flora

Determining the normal' viral flora of a bird is problematic, due to the difficulties involved in detecting the presence of any given virus, and then relating it to the disease process involved (if any). PBFDV detected in a lorikeet, for example, may indicate an active infection, a latent carrier, or simply a transient infection. Other viruses e.g. Bornavirus, may have become incorporated into the host's genome without causing disease (Horie et al., 2010), bringing into question the significance of a positive result in a PCR test.

Normal Parasitic Fauna

The dictionary definition of the word parasite is: *a plant or animal that lives on or in an organism of another species from which it derives sustenance or protection without benefit to, and usually with harmful effects on, the host*. The word parasite' is derived from the Greek “*parasitos*” - one who eats at the table of another. Parasite life cycles can be either direct (direct transmission from host to host) or indirect (where the parasite's life cycle includes a vector that carries it from one host to another). Wild birds frequently carry moderate loads of parasites, but rarely as severe as those seen in captive birds. Captivity - keeping an animal confined in a relatively small area - increases the exposure of birds to both direct and indirect parasitic life cycles.

Parasites of birds include helminths (nematodes, cestodes, and trematodes), protozoa, and

arthropods. Most, if not all, organ systems can be affected the skin, respiratory tract, gastrointestinal tract, kidneys, muscles and blood. Some parasitic infections can result in illness and death; other parasites are more successful', rarely affecting the host off which they live, and even conferring a degree of immunity onto the host. It has been shown, for example, that low pathogenic burdens of *Eimeria* spp in poultry can result in the development of immunity in these birds to more pathogenic infections (Jenkins et al., 2009). It follows, therefore, that the detection of some parasites in birds does not indicate the likelihood that these parasites are always detrimental to the host. However, none have a symbiotic relationship with birds, where both host and parasite gain from the relationship.

As the husbandry and nutrition of captive birds has improved, the incidence of parasitic infections has decreased. However, parasitic infections can be a serious health and management issue in some bird collections and individuals, and the clinician should always be alert to their presence.

SO, WHY DO BIRDS GET SICK?

Predisposing Factors in Infectious Diseases

As discussed earlier, infectious micro-organisms and organisms can be primary disease-causing agents or secondary invaders. Birds may be exposed to them through environmental contact, or they may be resident flora. Their ability to cause disease is determined by the interaction of 1) host factors and 2) pathogen factors.

Host factors include the state of the bird's defense system, its general health, the presence of concurrent disease, and external stressors. The defence system consists of both nonspecific defences (epithelial surfaces, normal flora, and leukocytes) and specific defences (the humoral immune system and the cell-mediated immune system).

The skin and the mucosal linings of the intestinal, respiratory, urinary and reproductive tracts are the first line of defence in preventing pathogen access to the body. In a healthy bird, this is achieved by providing both a physical barrier to pathogens and establishing a resident population of bacteria with a low pathogenicity (normal flora), thereby inhibiting the entry and growth of pathogenic micro-organisms. This normal flora takes up available space, occupies receptors and acts competitively against invaders by various mechanisms such as inhibitory metabolic products, bacteriocins and production of a low pH environment that inhibits the proliferation of gram-negative rods and yeast. (Gerlach, 1994; Harrison and McDonald, 2006). If pathogenic bacteria do succeed in colonising and penetrating this first line of defence, leukocytes are the body's first responders', identifying and phagocytosing bacteria that do not belong.

While nonspecific defences either deny pathogenic bacteria entry to the body or destroy them when they enter the body, specific defences play a prophylactic role by defending the body against initial and recurrent infections. The specific defence mechanism relies mainly on B- and T-lymphocytes to recognize antigens and to produce microorganism-specific antibodies (humoral immune system), or to provoke cell-mediated reactions (cell-mediated immune system). (Gerlach, 1994).

These defence mechanisms function best in a healthy bird; birds that are malnourished (protein, vitamin, and mineral deficient diets i.e. predominantly seed-based diets), suffering from concurrent disease (e.g. PBFD, herpesvirus infection, chlamydiosis, and mycotoxcosis) or are subjected to

repeated external stressors (overcrowding, noise, abnormal diurnal rhythms, etc) are less able to mount an effective defence against real or potential pathogens.

Pathogen factors include the virulence of the micro-organism in question and the concentration of pathogens to which the bird is exposed. These pathogens, once they have entered the body, have to survive the natural host defences and compete with the established non-pathogenic flora in order to become established. This requires the ability to colonise the site of infection, determined by the physical characteristics of the micro-organism (e.g. the presence/absence of flagellae), adherence factors and enzymes. Many pathogens also possess virulence factors that enable them to cause primary damage to targeted organs, such as invasion, secretion of cytotoxins or enterotoxins, and resistance to phagocytosis. (Batt, 2002).

Exposure to relatively low numbers of even virulent micro-organisms usually presents little challenge to a healthy bird with a well functioning immune system. Those same micro-organisms, allowed to concentrate in the bird's environment or food, can present the bird's natural defences and immune system with an overwhelming challenge, leading to infection. Good hygiene and appropriate storage of food is essential to reduce the concentration of potential pathogens the bird may be exposed.

It is the interaction of these host and pathogen factors that is the determinant of infection. Birds that are immunosupressed or immunocompromised for any reason, when challenged by potential pathogens, may rapidly succumb to infection. Mildly virulent pathogens, when met with an ineffective response from the non-specific and specific defence mechanisms, are able to colonise various entry portals and penetrate beyond them. On the other hand well nourished healthy birds, when challenged with a low to moderately virulent pathogens, are usually able to prevent this same colonisation and penetration.

DIAGNOSIS OF INFECTIONS

When revisiting Virchow's belief that pathogens "seek their natural habitat - diseased tissues - rather than causing disease" it follows that simply identifying the presence of abnormal or potentially viruses, pathogenic bacteria, fungi or other micro-organisms in a bird does not mean that an infection or disease process is present. These micro-organisms may be resident flora or simply transitioning through the bird after been ingested or inhaled. Determining whether these micro-organisms are playing a role in the bird's current health status is the "art of avian practice" (Rosenthal, 2006)

Traditionally veterinarians have employed antibiotics in the treatment of sick birds', especially when the subjective finding that the bird is ill is supported by the presence of Gram negative bacteria in a faecal or choanal swab. This approach had some validity in the earlier days of avian medicine when birds were housed poorly, fed a sub-optimal diet and poor hygiene was the order of the day - the opportunities for pathogenic bacteria to become secondary or even primary disease agents were much greater. Now most birds are fed better diets, housed more appropriately and the importance of good hygiene is well understood. The chances of birds coming into contact with significant levels of pathogens are less, and the bird's defence mechanisms are better. Infectious micro-organisms, both bacterial and viral, play a smaller role in disease than these organisms did 15 years ago. (Rosenthal, 2006)

In making an assessment of the significance of a pathogen identified in a bird by PCR, Gram stain or culture, the clinician must look beyond the laboratory report. (Fudge, 2001) A diagnosis of a primary

infection in an adult bird is almost always a disease of exclusion. If all other disease processes are ruled out, a primary infection may then be considered. (Rosenthal, 2006) The patient's history, physical findings, haemogram, clinical biochemistries and other diagnostic aids must be employed in making the final determination of the significance of microbiological findings. (Fudge, 2001; Rosenthal, 2006) If we accept Virchow's belief that pathogens are most commonly (always?) secondary invaders, the clinician's role in a diagnostic sense is to determine the primary problem that allowed these pathogens to colonise and penetrate the patient's body. In other words, why did this bird get sick?

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