Microbiology: Revisiting the Gram Stain and Culture

Karen L. Rosenthal

Bacterial infections can occur in all species of birds. Although perceived by some as a common problem, bacterial infections causing primary and even secondary disease are probably over-diagnosed in pet birds. Just because one can culture a bacteria from a bird does not mean it is the cause of a disease. Determining if a bacterium grown in culture actually is producing disease is the art of avian practice.

It is easy to culture any site in a bird and recover one or more bacterial species. The question remains, is a bird "healthy" when gram negative bacteria are cultured even if the patient appears clinically normal or does the number of bacteria found equate to disease or do we interpret cultures primarily in light of clinical signs? In a clinically healthy bird, a choanal culture may only characterize bacteria found in the environment and not necessarily disease causing pathogens. Routine cultures of the choana and/or cloaca may make more sense in a flock than in an individual bird. In an aviary, routine cultures of selected individuals may give the veterinarian a sense of the potential pathogens in that flock. Routine environmental bacterial culturing may also be necessary.

Previously, it had been assumed that bacterial infections were a large part of the diseases processes that attacked pet birds. There are many reasons why this is no longer true. The signalment of pet birds presented to veterinarians has changed over the years. Twenty years ago, most pet birds were wild caught with suspect husbandry at best. Today, almost all pet birds purchased are domestically bred. Infectious disease, both bacterial and viral, play a smaller role in disease than these organisms did 15 years ago. Owners have more invested in these pets and have improved their husbandry. Cages are improved and diet is vastly improved. Owners have more information through books and the internet on how to properly care for their feathered pets. It is less common than in previous years to find caged birds living in conditions where they are constantly in contact with wastes. Owners are more aware that food prepared for the bird can become contaminated in the kitchen area before it is even given to the bird. Contaminated knives, feeding bowls, and cutting boards all can spread potential pathogens to birds.

What needs to be most discouraged in avian medicine today is the diagnosis of a bacterial infection because the bird is “sick.” This idea is leftover from 20 years ago when little in diagnostics and resources were available to the avian veterinarian. It may be tempting to reach for antimicrobials when a sick bird enters the office, especially when the owner does not want to spend any money on diagnostics. This should be discouraged. An adult sick bird is much less likely to be suffering from a primary bacterial infection that will be cured only with the administration of an antibiotic. Even if a respiratory infection is present, this may be secondary to poor husbandry (decreased humidity) and the infection will recur even with proper antibiotics if the primary cause of disease is not determined. A primary bacterial infection in an adult psittacine is almost always a disease of exclusion. If all other disease processes are ruled out, a primary bacterial infection may then be considered.

Contrary to what many believe, it is not possible to make a diagnosis of a bacterial infection in most birds with just a gram stain and a culture. There are many other diagnostic factors that are considered. Before a diagnosis of a bacterial infection can be made, it is important to recommend a minimum database of information on the patient. Although personal preferences may change what is included, a complete blood count and biochemistry profile should always be part of the database. This is important to determine if an infection is present. In almost all cases of bacterial infections, the white blood cell count should be elevated. In most cases, it is a heterophilic response. In severe, acute infections, band cells may be present, as they are in mammals. In chronic bacterial infections, it is common to find a mild non-regenerative anemia along with an increase in the monocytes. The biochemistry profile is not always helpful with the diagnosis of a bacterial infection but can be helpful in specific disease states. Both bacterial hepatitis and bacterial nephritis will likely cause elevations in hepatic and renal values, respectively. Chronic bacterial disease can change the total protein concentration. If enough antigenic stimulation is present, the protein value may increase. If proteins are consumed in the infection or are lost due to a chronic intestinal disease, the protein concentration may be lower. The protein electrophoresis may be useful to better elucidate the protein values. Another key to the diagnosis of a bacterial infection is the history. Does the bird live in a filthy environment? Is there possible food contamination? Is the bird prone to respiratory diseases during the winter or summer? Another important piece to the diagnostic puzzle is the signalment. Is this a neonate or unweaned chick that is not being properly cared for? The signs may also lead to a diagnosis. Is nasal discharge or sneezing present or is this just a non-specifically sick bird? So before a culture or gram stain is even attempted, ideally, the history, signalment, signs, and minimum database have all been scrutinized to determine if an infection should be considered.

So, contrary to popular opinion, the diagnosis of a bacterial infection does not necessarily start with a culture and gram stain but ends with those two tests. The gram stain is one of the most overused and abused diagnostic test in avian medicine. Properly done, a gram stain will give information on the percentage of gram negative to gram positive organisms in the particular location that was swabbed. That is the extent of the information that is revealed about a gram stain, in terms of gram negative and gram positive organisms. Whatever the meaning of those results might be, it is up to the avian veterinarian to determine the significance. The gram stain of a non-specific sick bird, no matter what orifice it is from, likely has no relation to the disease process causing the illness. It makes little sense in most cases to do a cloacal or choanal gram stain in almost every case of a sick bird, no matter the signs. Gram stains of abscess material or gram stains taken from distinct areas of infection give the veterinarian an initial clue of what the infection is and the likely antibiotics that would be successful.

With this in mind, a culture of a gram positive from the cloaca, the choanal, or the nares probably cannot be interpreted when trying to determine if this organism is causing disease. There are better ways to use the client's money. But what if these types of cultures are done and unexpected organisms are grown. This may include gram negatives such as *Klebsiella*, *Pseudomonas*, *Proteus*, to name a few. These organisms can cause severe disease and can be difficult to treat. There are some who would recommend treating the patient for these organisms even if you are not certain these organisms are causing disease. The research to support this argument is not available. Therefore, each case should be evaluated on its own merits. If a bird is compromised and a Klebsiella is identified, it may be worthwhile to treat for it keeping in mind that this may not be the primary problem of the patient. Species differences do exist. It is more common to find gram negative organisms in cockatoos or cockatiels, especially when culturing the cloaca. In most cases, those organisms do not need to be treated. Also, birds on different foods will have different flora in their gastrointestinal tract. What makes interpretation difficult for the avian veterinarian is the

inability to know what the normal flora is in pet birds. That is why other diagnostic tests and history are so important in being able to properly interpret culture results.

What if one of the newer drug resistant organisms is cultured such as a Multidrug Resistant (MDR) E. coli or a Methicillin Resistant Staph aureus (MRSA)? Again, it is not clear what to do with these organisms. It is known that people can carry MRSA and never break with infection. Less is known in birds about MDR E. coli and MRSA. In these instances, not only should treatment be considered but also the possibility of a nosocomial infection should be ruled out.

Microbiology testing can be done in house or sent to a microbiology laboratory. Unless the clinician and the staff are proficient at microbiology, it is best to send samples to a qualified laboratory. The outside laboratory should provide the veterinarian with supplies to do microbiologic cultures. The most common type of device is the culturette, including minitip, which consists of a swab that, after use, is placed into a transport media. One way to improve the chances of the culturette capturing bacteria is to moisten the swab with sterile saline before taking the sample. It is suggested the best way to improve the chances of not getting a "no growth" is to take the culture and then leave the culturette out at room temperature for one hour. After one hour, place the culturette in the refrigerator and keep the sample cool until it is in the laboratory. Pediatric blood cultures are ideal for birds and then can be used both for aerobic and anaerobic cultures. If an aspirate is collected, it should be placed into a culturette or into a transport tube if one is provided. Anaerobic cultures are under utilized in avian medicine. There are numerous types of sample containers for anaerobic cultures and the veterinarian is advised to call their laboratory to determine how best to send those samples. *Chlamydophila*, *Mycoplasma* and mycobacteria all require special handling to be cultured. The avian veterinarian needs to have contacts with laboratories equipped to culture these unusual organisms.

Bacterial infections in unweaned birds are probably more common than in any other age group. This is due to poor husbandry and the lack of a properly developed immune system. Crop infections are most prevalent due to incorrect hand feeding practices. Such practices as over feeding, feeding too frequently, improper formula temperature, or feeding before the crop empties can all lead to bacterial overgrowth. Primary viral infections destroying the immune system underlie severe secondary bacterial infections in young birds. Spontaneous, primary bacterial infections are uncommon in young birds when proper husbandry is practiced.

Crop sampling for bacterial cultures is indicated for any bird with crop stasis. In adult birds, a primary bacterial infection leading to crop stasis is rare. It is difficult to place the culturette in the crop without touching it against oral cavity tissues. A better method would be to retrieve material via a crop wash with sterile saline. It is best to also do a cytology and wet mount on the crop wash. It is expected in a normal crop to culture small numbers of gram positive organisms. In a diseased crop, heavy growth of gram negatives is not uncommon. Even if a primary problem, such as renal disease has led to crop stasis, a secondary crop infection should not be ignored and needs to be treated.

Respiratory disease is not uncommon in pet birds. The frequency in which this disease is due to bacterial infections is unknown. Upper respiratory disease signs may be due to a bacterial infection, fungal infection, or non-infectious causes. The choana is the repository for environmental bacteria breathed in by the bird and as such, a culture of this area represent the environmentals and not necessarily bacterial pathogens. Nasal discharges should also never be used as a culture site. They are heavily contaminated with environmental organisms and would be impossible to interpret as to whether they are pathogenic. Common upper respiratory inhabitants

include gram positive organisms such as Streptococcus spp, Staphylococcus spp. and Enterococcus spp. These organisms, if obtained from a sterile culture from a sinus, could be pathogenic. More likely pathogenic organisms of the respiratory tract would include Pseudomonas, Aeromonas, Klebsiella, and Pasteurella.

Samples for upper respiratory bacterial infections are best cultured from the sinuses. If a sinus trephination is performed, material can be cultured from the surgical site. A less invasive method is to aspirate material from a sinus or collect material for culture from a sinus flush using sterile saline. These samples should be sterile in a healthy bird so any bacteria or fungus cultured from these methods is significant. These are common areas for Chlamydophila and mycoplasma infections and no growth on typical bacterial media may indicate an infection with one of those organisms. Lower respiratory infections are usually bacterial and/or fungal in origin. Tracheal samples can be obtained directly by swabbing the trachea, or by endoscopic means or from a tracheal wash. Other areas to sample with lower respiratory disease include the lungs and air sacs. These samples should be sterile in a healthy bird so any bacteria or fungus cultured from these methods is significant.

The irony about bacterial cultures in birds is that they are the easiest diagnostic test to perform. But they are the most difficult diagnostic test to interpret. The avian veterinarian is encouraged not to reach for the culturette and antibiotic immediately upon presentation of a sick bird but to use other diagnostic methods to rule in or out a bacterial infection.