WHAT'S THE VALUE OF THAT TEST? USING SCIENCE TO TAKE A CLOSER LOOK AT COMMON AVIAN DIAGNOSTIC TESTS

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Veterinarians routinely order up diagnostic tests to help rule-in or rule-out diseases on a differential list, and it is common for clinicians to accept these results on face value. However, it is important to consider that the results may be biased because of the methods used, type of sample, or inherent limitations of the assay. Considering these limitations is important to help minimize the likelihood of misinterpreting these results. Several common tests used to screen avian patients, including Gram stains, culture, and haematology, are subject to these potential biases. This is especially common with these types of tests because of the natural variances associated with them and a lack of rigorous methods for evaluating their true meaning. The purpose of this presentation is to use statistics to help us better understand the value of these common tests. Reviewing these limitations using "real world" examples will help prepare the clinician to better interpret the tests they rely on when managing their cases.

Faecal Gram stains are commonly performed as a method of evaluating the gastrointestinal health of psittacine species. In birds presenting with evidence of clinical disease, Gram stains afford timely, inexpensive information regarding the general classification of potential pathogens (e.g., Gram negative; Gram positive spore forming bacteria) and provide some information if culture results are unobtainable. In practice, Gram stains are commonly used by some clinicians as part of avian wellness exams. However, a disadvantage of using Gram stains to evaluate clinically healthy birds is that there is not always good agreement between the Gram stain results and the status of the patient. In cases where there is clinical disease, cloacal/faecal bacterial cultures are often used in combination with abnormal Gram stain results to assess the patient; however, organisms found on Gram stain might not be isolated on culture. Because there are no formal studies evaluating the "correctness" of the results, clinicians are often left wondering how to interpret the results. In these types of latent cases (e.g., not sure the patient's true status), we can use agreement analysis to interpret the results. For categorical data (e.g., presence/absence), kappa analysis is recommended. In cases where the data is ordinal or ranked data (e.g., none, few, many), a weighted kappa works best. For continuous data (e.g., bacterial counts; proportions), Bland Altman analysis and Passing Bablok regression can be used. These types of statistic allow use to use data available in our own clinics and based on the specific methods we use to interpret the meaning of the tests. A review of these methods and how to perform and interpret them will be covered in the presentation. In the author's practice, the level of agreement between culture and Gram stain is considered fair. Knowing this limitation has been useful for pursuing cases when clinical disease was/was not present in an avian patient.

The complete blood count is another common avian diagnostic test that veterinarians rely on when making decisions about the physiologic and immunologic status of a patient. Because all blood cells are nucleated in birds, it is not possible to rely on the same haematologic equipment used for mammals. Instead, veterinarians must rely on semi-quantitative methods for estimating white blood cell counts in these animals. The common methods used are based on estimates performed off of a blood smear or a combination of the differential count from the blood smear and a semi-quantitative cell count using a haemocytometer and special stain (e.g.,

phloxine B). In the USA, clinics rely on both of these methods; some utilize one or the other, while some use a hybrid technique depending on personnel and time. Since the management of a case often strongly relies on these results (e.g., inflammation or no inflammation; antibiotics or no antibiotics), it is important for the clinician to recognize the limitations of this test and learn how to manage these potential distractors. From how the sample was collected and stored to the method used to make the smear and stain the slide; all of these factors can influence the result and the direction a case may be pursued. As with the Gram stain-culture example, agreement analysis can be used to determine the relative value of complete blood cell counts being performed at a veterinarian's facility or at a laboratory to provide insight into what is otherwise latent data. Once these parameters are defined, it simplifies the process of deciding when problems with the testing method, rather than the patient, arise.

When interpreting complete blood counts, it is important to consider the data in a relative versus absolute fashion. For some this may be difficult, as veterinarians are trained on "normals". "Normal" ranges that are provided by a laboratory or found in a reference rely on absolute boundaries; however, they are collected at a population versus individual level. Inherent individual variance and wide distributions in references can be misleading. The only time the author finds it appropriate to rely on absolute data is when the patient is evaluated against its own historic data. It is for this reason that collecting annual blood work on our patients is important. Being able to establish a specific reference for the individual animal that can be used to assess percent change in certain parameters is quite helpful for determining the status of a patient when it is clinically abnormal.

Statistics are often considered taboo by veterinarians; however, they are an essential "diagnostic" that can assist us with determining the importance and reliability of the clinical diagnostic tests we use to assess our patients. With a little practice, clinicians can develop in-house references to determine the relative value of the common avian diagnostic tests they use based on the specific methods used in that practice or laboratory. By doing so, veterinarians will refine their ability to use these tests and improve their clinical efficiency and success.