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Laboratory Reporting Accuracy of Polymerase Chain Reaction Testing for Psittacine Beak and Feather Disease Virus

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Abstract: Diagnostic assays that use polymerase chain reaction (PCR) methods are increasingly available to veterinarians. Psittacine beak and feather disease virus (family Circoviridae, genus *Circovirus*) is a pathogen of clinical importance for which PCR assays have been developed. Several laboratories offer this diagnostic assay in the United States; however, there is little information on the sensitivity, specificity, or accuracy of these assays. In this study, known positive (n = 10) and negative (n = 10) samples were sent to 5 commercial laboratories. Accuracy was 100% for 2 laboratories, 95% for 2 laboratories, and 71% for 1 laboratory; the accuracy of the latter laboratory was affected because of a specificity of 20%. These results suggest that although the results from most laboratories are highly accurate, both false-positive and false-negative results are occasionally reported by at least 2 laboratories. These results also suggest that at least 1 laboratory may be generating large numbers of false-positive results.

Key words: psittacine beak and feather disease virus, circovirus, polymerase chain reaction, PCR, diagnostic tests, avian

Introduction

Polymerase chain reaction (PCR) is one of the most important diagnostic tools developed in the past 20 years for identifying and characterizing pathogens. With PCR assays, low copy numbers of DNA or RNA can be amplified to levels that can be detected. This technique involves amplifying known sections of RNA or DNA to detectable concentrations that can be measured visually. New techniques are constantly being improved and updated. Many techniques have been published for identifying pathogens, but there is little to no independent validation of the commercial laboratories that offer these diagnostic tests on avian samples.

With PCR assays, DNA is extracted from a sample and then is heated with a heat-resistant DNA polymerase, a buffer, and known DNA primers. The primers are selected so they bind a known distance apart to opposite ends of the viral DNA. If viral DNA is present, repeated heating and cooling of the DNA fragment allow amplification to a detectable concentration. Blood, feces, tissue, exudates, or environmental samples can all be examined for small quantities of DNA or RNA.[1]

In human medicine, PCR-based diagnostic assays must undergo rigorous testing before being implemented in a clinical laboratory. According to the Clinical Laboratory Improvement Amendments of 2004, a new PCR-based test must be from either a US Food and Drug Administration–regulated kit or an in-house developed test that has been properly validated.[2] According to the Clinical and Laboratory Standards Institute, validation requires that the testing modality meets user needs by ensuring quality control, proficiency testing, validation of employee competency, instrument calibration, and correlation with clinical findings.[3] There are no similar guidelines for these criteria required for PCR diagnostic tests in veterinary laboratories.

Psittacine beak and feather disease (PBFD) is caused by a circovirus known as beak and feather disease virus (BFDV).[4] This virus is in the genus

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Circovirus, which, along with the genus *Gyrovirus*, make up the family Circoviridae.[5] Circoviridae are all enveloped, single-stranded DNA viruses. The *Gyrovirus* genus consists of the chicken anemia virus, which shares no significant genotypic similarity with BFDV.[6] Psittacine BFDV is 1 of 4 members of the genus *Circovirus* recognized by the International Committee on the Taxonomy of Viruses[4] that affect the class Aves: BFDV,[6] canary circovirus,[7] pigeon circovirus,[8] and goose circovirus.[8]

Psittacine BFDV is an important pathogen in many groups of captive and wild birds globally. As such, positive or negative results of diagnostic tests can have significant clinical effect when individual birds, populations, or their environments are tested. The PCR technology for detecting BFDV has been established in both clinical and research settings, but many different specific PCR tests and methodologies are available.[9–13] Genotypic variability of BFDV has also been reported[10,11,13] and could result in failure of primer annealing, resulting in false-negative results. The challenge for practitioners is not only to choose the most appropriate test for screening their patient but also to determine which laboratory will provide the most accurate result.

Several veterinary laboratories offer psittacine BFDV testing on avian samples. To our knowledge, there are no governmental or professional guidelines that require standardization or validation of testing methodologies before use in a commercial setting. Veterinary laboratory validation for PCR testing is a voluntary method to ensure minimum quality standards. No overseeing entity validates PCR techniques specific to avian medicine, which leaves the practitioner with no means to compare laboratory sensitivity and specificity. Reports of in-house specificity and sensitivity testing for BFDV in a commercial laboratory are minimal.[14] No comparison study has been published, to our knowledge, to evaluate differences among laboratories and the results generated for their customers. Usually, clinical laboratories are selected by practicing veterinarians based on familiarity with the laboratory rather than on a factual understanding of the validation or sensitivity and specificity of the tests they are requesting.

This clinical study focused on the use of PCR testing for a specific pathogen, BFDV, and the potential result variability among laboratories for both known-positive and known-negative samples.

Materials and Methods

A BFDV infection was confirmed in a 3-month-old Meyer's parrot (*Poicephalus meyeri*) that died. Diagnosis was based on clinical signs (dystrophic feathers and severe loss of contour feathers); the presence of viral DNA in a whole-blood sample, as determined by PCR testing; the presence of microscopic lesions (large numbers of histiocytic cells containing intracytoplasmic inclusions typical for circovirus); and positive results of immunohistochemical staining from the bursa of Fabricius and feather follicles. Liver samples from this bird were used as positive samples. Negative samples were obtained from specific-pathogen–free (SPF) day-old chicks (Poultry Laboratory, University of California, Davis, CA, USA).

A total of 10 positive and 10 negative samples were prepared for each laboratory in the study. Aseptic technique was used to collect and process samples, all of which were processed on the same day. Positive samples were prepared from liver tissue collected from the confirmed BFDV-positive bird. Negative samples were prepared from liver samples from SPF chicks. For both positive and negative samples, a liver homogenate was made with 1 g of liver tissue by macerating it with sterile slides and mixing it into 5 ml of 0.9% sterile saline with sterile applicators. Sterile swabs were used to collect 5 positive and 5 negative samples for each laboratory. For diluted samples, a single dilution of a 0.1-ml aliquot of the original homogenate was mixed with 99.9 ml of 0.9% sterile saline, producing a 1 : 1000 dilution. A total of 5 positive and 5 negative samples of the dilution were collected for each laboratory with sterile swabs. All samples were assigned a randomized, individual identifying number-letter combination. One set of known-positive (5 undiluted, 5 diluted) and 1 set of known-negative (5 undiluted, 5 diluted) samples were packed according to laboratory-specific packaging methods and shipped to 5 individual laboratories for PCR testing. All results were recorded as positive or negative only.

The laboratories that received samples were selected empirically based on their ability to perform commercial BFDV PCR testing. Data were evaluated on an individual and an overall, combined laboratory basis.

The sensitivity, specificity, and accuracy of reported results were calculated. By definition, sensitivity refers to the proportion of individuals with a disease who are correctly identified by a test, whereas specificity refers to the proportion of individuals without the disease who are correctly

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Table 1. Test results reported from 5 veterinary diagnostic laboratories from known positive (n = 10) and negative (n = 10) samples submitted for PCR testing for psittacine BFDV.

Laboratory	Incorrect results (n)	Correct results (n)	Accuracy, %
A	8	12	71
B	1	19	95
C	0	20	100
D	0	18	100
E	1	19	95
Totals	10	88	91

identified by the test. Sensitivity [*sensitivity = true positive*/(*true positive + false negative*)],[15] specificity [*specificity = true negative*/(*true negative + false positive*)],[15] and accuracy [*accuracy* = (*true positive + true negative*)/(*true positive + false positive + false negative + true negative*)] were calculated for each laboratory and for all laboratories collectively. Authors were blinded as to the identity of the laboratories providing specific results.

Results

Results of PCR testing by the 5 laboratories are summarized (Tables 1 and 2). Results show 4 of the 5 laboratories evaluated (80%) had a 95% or greater accuracy when reporting the presence or absence of BFDV in each sample.

Two results from laboratory D were discarded because of being mislabeled. Both samples were labeled with the same identifier and were originally reported from the laboratory as a positive sample and a negative sample. Individual costs of laboratory fees ranged from $15 to $65 per test, not including shipping fees. Reporting times ranged from 2 to 10 days.

Discussion

In an ideal world, test results from samples containing a sufficient number of organisms would be expected to be positive, whereas those from samples that did not contain organisms would be negative, if tested by a validated PCR assay. However, sampling error, sample degradation in transport, human error, and poor quality-control practices at the laboratory can result in less than 100% accuracy. This study was done to compare the individual accuracy of 5 laboratories, as well as the overall group accuracy, by using samples that were known positive and known negative for BFDV. Our results show that, based on a sample of 5 laboratories offering PCR testing for BFDV, most laboratories evaluated were consistent with a practitioner's general expectations or clinical need. Of the laboratories in this study, 2 of 5 (40%) had 100% accurate results; 2 of 5 (40%) reported 95% accuracy; and 1 of 5 (20%) reported 71% accuracy. Overall combined laboratory sensitivity (98%) was well within acceptable limits to meet the needs of most practitioners. Specificity (82%) was also within acceptable limits for many diagnostic testing protocols. Individual laboratory sensitivity ranged from 90% to 100%, and specificity ranged from 20% to 100%.

These findings have both scientific and practical implications. Laboratories A and E have less than 100% specificity (20% and 90%), resulting in more samples reported as positive than were truly positive. Potential implications for aviary and pet stores are that these results may cause an increased number of birds to be euthanatized because of fear of an infectious disease that is not truly present. Additionally, false-positive tests can result in increased medical costs, death of an animal, damage to the reputation of the retailer, or potential loss of the practitioner's reputation if the results are later proven to be false. Laboratory B had a less than 100% sensitivity, which could result in positive samples being reported as negative. In an aviary or pet store setting, this could have important consequences if positive birds are inaccurately reported and allowed into a

Table 2. Sensitivity and specificity of polymerase chain reaction tests for beak and feather disease virus from 5 veterinary laboratories. Known true-positive (n = 10) and true-negative (n = 10) samples were submitted to each laboratory.

Laboratory	Test positive	Test negative	Sensitivity, %	Specificity, %
A	18	2	100	20
B	9	11	90	100
C	10	10	100	100
D	9	9	100	100
E	11	9	100	90
Totals	57	41	98	82

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naive population. The cost of the test could also play a role in laboratory selection. In our study, cost was an important factor in determining the sample size per laboratory. In this study, we did not evaluate accuracy compared with cost.

Several weaknesses are present in a study of this small size. The relatively low sample size limits the ability to make strong statistical statements from the data. Our results are at least suggestive of questionable accuracy among some of the laboratories offering PCR diagnostic testing. If our results are representative of the accuracy of each laboratory, then 3 of the 5 laboratories evaluated (60%) reported erroneous results with possible clinical implications. With a larger sample size, it is likely that none of the laboratories evaluated would have reported 100% accuracy.

Sample-handling error in collecting samples and at the laboratory can result in the reporting of inaccurate results. Environmental contaminants are most likely to result in false-positive results, whereas improperly shipped samples may be reported inaccurately positive or negative. Labeling errors can occur with all diagnostic samples, which may play an important role in determining the fate of a bird suspected or being infected. Additionally, genotypic variability of BFDV may result in positive sample results being inaccurately reported as negative.

The liver tissue from the SPF chickens used in this study was not evaluated histologically or by immunohistochemical staining for evidence of circoviral infection. Natural infection of poultry with psittacine BFDV has not been reported. If BFDV had been present in our SPF samples, we anticipate that it would have been more equally reported by all laboratories. Specifically, chicken anemia virus was not screened in these samples before use in this study because of the lack of conserved regions between these 2 viruses.[6]

The focus of this study was on the practitioner's perspective, rather than to evaluate specifically the scientific validity of BFDV PCR technology used by these laboratories. This study was designed as an attempt to objectively compare laboratory accuracy both individually and collectively. Our research has shown that results from most of the laboratories evaluated were within acceptable limits (sensitivity and specificity) for the needs of most avian practitioners when evaluating samples for the presence of BFDV. However, the range in overall accuracy observed (71%–100%) could be a clinically important finding, should a larger study corroborate our findings.

Quality control and objective validation of techniques within laboratories is voluntary. Many practitioners tend to assume that laboratories execute quality control measures equally. The findings from this study will, hopefully, stimulate an increased effort in voluntary validation of methods used by individual laboratories. The publishing of assay sensitivity and specificity values would greatly assist practitioners in choosing an appropriate diagnostic laboratory. In addition, the inclusion of third-party involvement in quality control and oversight of PCR diagnostic testing should improve the ability of veterinarians to accurately interpret the reported test results. Laboratory choice by practitioners is usually based on personal experience, blind faith, or cost. Objective third-party evaluation of each laboratory's methods would allow practitioners a more solid foundation on which to base these important decisions. Our research was only a small study and has not been evaluated for the repeatability of its results.

The true risk of receiving inaccurate test results from laboratories that perform PCR tests for psittacine BFDV is undefined at this time. Our initial results support a need for further research with much larger sample sizes and the inclusion of additional infectious organisms. Ultimately, practitioners remain primarily responsible for interpreting laboratory results rather than simply acting on the results as if they contained absolutely infallible data.

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References

1. Phalen DN. Avian viral diagnostics. In: Fudge AM, ed. *Laboratory Medicine: Avian and Exotic Pets*. Philadelphia, PA: WB Saunders; 2000:111–123.
2. Clinical laboratory improvement amendments: current CLIA regulations (including all changes through 01/24/2004). Centers for Disease Control and Prevention Web site. http://www.phppo.cdc.gov/clia/regs. Accessed May 6, 2007.
3. Clinical and Laboratory Standards Institute. Harmonized terminology database. CLSI Web site. http://www.clsi.org/source/custom/termsall.cfm. Accessed May 4, 2007.
4. International Committee on Taxonomy of Viruses. ICTVdB index of viruses Web site. http://www.

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ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_circo.htm. Accessed May 11, 2007.

5. Johne R, Fernandez-de-Luco D, Hofle U, Muller H. Genome of a novel circovirus of starlings, amplified by multiply primed rolling-circle amplification. *J Gen Virol*. 2006;87:1189–1195.
6. Bassami MR, Berryman D, Wilcox GE, Raidal SR. Psittacine beak and feather disease virus nucleotide sequence analysis and its relationship to porcine circovirus, plant circovirus, and chicken anaemia virus. *Virology*. 1998;249:453–459.
7. Phenix KV, Weston JH, Ypelaar I, et al. Nucleotide sequence analysis of a novel circovirus of canaries and its relationship to other members of the genus *Circovirus* of the family Circoviridae. *J Gen Virol*. 2001;82:2805–2809.
8. Todd D, Weston JH, Soike D, Smyth JA. Genome sequence determinations and analyses of novel circoviruses from goose and pigeon. *Virology*. 2001;286:354–362.
9. Ypelaar I, Bassami MR, Wilcox GE, Raidal SR. A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. *Vet Microbiol*. 1999;68:141–148.
10. Heath L, Martin DP, Warburton L, et al. Evidence of unique genotypes of beak and feather disease virus in southern Africa. *J Virol*. 2004;78:9277–9284.
11. Bassami MR, Ypelaar I, Berryman D, et al. Genetic diversity of beak and feather disease virus detected in psittacine species in Australia. *Virology*. 2001;279:392–400.
12. Ogawa H, Yamaguchi T, Fukushi H. Duplex shuttle PCR for differential diagnosis of budgerigar fledgling disease and psittacine beak and feather disease. *Microbiol Immunol*. 2005;49:227–237.
13. Hsu CM, Ko CY, Tsaia HJ. Detection and sequence analysis of avian polyomavirus and psittacine beak and feather disease virus from psittacine birds in Taiwan. *Avian Dis*. 2006;50:348–353.
14. Dahlhausen B, Radabaugh S. Update on psittacine beak and feather disease and avian polyomavirus testing. *Proc Annu Conf Assoc Avian Vet*. 1993:5–7.
15. Altman DG, Bland JM. Statistic notes—diagnostic tests 1: sensitivity and specificity. *BMJ*. 1994;308:1552.

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