UNIVERSIDAD DE SAN CARLOS DE GUATEMALA

FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA

ESCUELA DE MEDICINA VETERINARIA

MUESTREO DEL VIRUS DE LA ENFERMEDAD DE PICO Y PLUMAS (BFDV) EN PSITTACINOS NEOTROPICALES DE GUATEMALA DEL COMERCIO ILEGAL DE VIDA SILVESTRE CONSIDERADOS PARA REHABILITACIÓN Y REINTRODUCCIÓN

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SURVEY OF BEAK AND FEATHER DISEASE VIRUS (BFDV) IN GUATEMALAN NEOTROPICAL PSITTACINES FROM ILLEGAL WILDLIFE TRADE CONSIDERED FOR REHABILITATION AND REINTRODUCTION

ROXANA XIMENA SIBRIAN URRUTIA

MÉDICA VETERINARIA

GUATEMALA, SEPTIEMBRE DE 2021

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ACTO QUE DEDICO A:

A Ricardo Sibrián: Por ser la persona que me apoyo en todo el camino, confió en mí y me dio su apoyo en todo momento

A mis hermanos: Por su amor incondicional, su apoyo infinito y su fe en mis capacidades

A mis mentores: Por demostrarme lo que es tener pasión profesional y integridad personal y por compartir su sabiduría conmigo

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JOURNAL OF AVIAN MEDICINE AND SURGERY

October 19, 2020

Ms. Ximena Sibrián
Universidad de San Carlos de Guatemala Facultad de Medicina Veterinaria y Zootecnia
Wildlife
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GUATEMALA

Dear Mr. Sibrián:

This is a confirmation that your manuscript, AVIANMS-D-20-00042R3, “Survey of Beak and Feather Disease Virus (BFDV) in Guatemalan neotropical psittacines from illegal wildlife trade considered for rehabilitation and reintroduction” co-authored by Alejandro Morales DVM, MSc, Ximena Sibrián, Flor D. Porras DVM, has been accepted for publication in *the Journal of Avian Medicine and Surgery*. This manuscript will be published in a 2020 issue of the *Journal of Avian Medicine and Surgery*.

With Regards,

Thomas N. Tully, Jr., DVM, MS, DABVP (Avian), DECZM (Avian)
Scientific Editor *Journal of Avian Medicine and Surgery*

Running Head: BFDV IN NEOTROPICAL PSITTACINES IN REHABILITATION PROGRAMS

Survey of Beak and Feather Disease Virus (BFDV) in Guatemalan neotropical psittacines from illegal wildlife trade considered for rehabilitation and reintroduction

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Abstract: Beak and Feather Disease Virus (BFDV) is the causative agent of Psittacine Beak and Feather Disease (PBFD), a progressive and often fatal disease in birds from the order Psittaciformes. Even though neotropical psittacine species are more resistant to clinical infection than Old World species, Beak and Feather Disease Virus (BFDV) is recognized as a threat to immunologically naïve wild psittacine flocks and its epidemiological control is paramount for conservation efforts in neotropical species. Samples were collected from multiple psittacine species, including *Ara* species, *Amazona* species and *Pionus senilis* from the only rescue center in Guatemala with formal psittacine rehabilitation and reintroduction programs. A total of 117 birds were tested for BFDV by means of a real-time PCR assay. The BFDV prevalence found in this study was 0%, [CI 95%, 0% - 6.0%]. Seven 2 - 8 years old *Ara macao* with positive results from previous surveys via conventional PCR, yielded negative results in this study, suggesting complete infection resolution. This is the first report of a BFDV survey in Guatemala.

Key words: BFDV screening, Psittacine circovirus, *Amazona*, *Ara*, *Pionus*, *avian*, conservation, reintroduction

Introduction

Beak and Feather Disease Virus (BFDV) is a DNA virus of the *Circoviridae* family, with tropism for epithelial cells of various tissues, especially skin and gastrointestinal mucosa.[1,2,3,4,5,6] Psittacine Beak and Feather Disease (PBFD) is a progressive disease that causes abnormal beak and feather growth, alopecic areas, immunosuppression and death in birds, and its causative pathogen is BFDV.[1,2,7,8,9,10,11,12] All species from the order Psittaciformes, including Neotropical psittacine species, are considered susceptible to BFDV infection and can develop PBFD.[13] However, Neotropical psittacines seem to be more resistant to establishing an infection than Old World psittacines, even to the same viral strain, and once infected develop PBFD less frequently.[3,4,14,15] Non psittacine species, like columbids, corvids and raptors, also seem to be affected by BFDV, with cases reported in ecosystems with high circoviral prevalence in psittacine species.[16]

Currently BFDV has been reported in more than 60 psittacine species worldwide among pet and wild birds. This wide distribution of the disease has been strongly driven by the increase in both legal and illegal pet bird traffic in recent decades.[3,4,8,14,17] BFDV is recognized as a threat to wild populations of endangered species in Australia, New Zealand, and South Africa.[1,2,3,7,17,18,19] At the moment BFDV has not been reported in neotropical psittacine species of the American continent in the wild.[4,7,17] However, there are reports of the virus presence in captive birds, both native and exotic species, in households, shelters and rescue centers in Costa Rica, Brazil and Chile.[3,8,9,20]

BFDV detection in avian samples does not always imply PBFD development. Infection response varies between avian species and the disease presentation is associated with factors such as young age, immunosuppression or concomitant diseases.[3,4,5,9] PBFD development can be acute and deadly in some species, such as young cockatoos and African Grey Parrots. While most neotropical parrots and macaws show resistance to developing PBFD when naturally infected with BFDV and succeed in acquiring immunity,[11,14,18,21] removing the virus from their system after several months of transient infection.[11,12,14,19,21] Most PBFD cases reported in neotropical species were chicks with characteristic feather lesions that recovered spontaneously, turned from initial viral DNA positive tests to negative results in blood and feathers and afterwards grew new normal feathers.[21]

Although it is unlikely that PBFD would develop in all BFDV carriers and could extinguish a species in its natural habitat by itself, it can negatively impact attempts to recover endangered species by reducing fitness and survival chances of reintroduced birds and infecting wild born nestlings.[2,17,18,22,23,24] Outbreaks in wild Old World psittacine flocks have been associated with the release, intentionally or accidentally, of a BFDV carrier into their ecosystem, usually exotic pet birds.[7,13,16,17,18,20]

Illegally trafficked wild birds present higher risk of contracting and transmitting infectious diseases than captive born birds due to exposure to other carrier species, stress induced immunosuppression and poor animal care.[3,7,25,26] Since most parrot owners, including Latin-American ones, generally disregard basic care of the species they have acquired, wild birds kept as pets are unknowingly mishandled and suffer nutritional

deficiencies, stress and immunosuppression, which may lead to higher susceptibility to viral infections.[19,25]

Contamination can be persistent and hard to eradicate from aviaries[12] due to the remarkable resistance of BFDV to environmental conditions[17,18,22,23,27], inactivation by high temperatures, most disinfectants and cleaning products.[2,21] Infected birds can spread the virus through feathers, feather dust and feces, contaminated cages, perches, food and water bowls in captivity, and tree cavities and nests in the wild. [2,16,23] Since smuggled birds and illegal household pet birds are rescued with the intention of rehabilitation and/or reproduction for reintroduction programs, ruling out the presence of BFDV in captive flocks in rescue centers and shelters is of paramount importance due to the unknown impact of introducing an exotic pathogen in the immunologically naïve wild populations and the possible interspecies interactions.[1,7,9,23,27]

Currently, the only wildlife rescue center in Guatemala that rehabilitates and reintroduces native psittacine species into its natural habitat is the Wildlife Rescue and Rehabilitation Center of the Wildlife Rescue and Conservation Association (ARCAS), in the department of Petén, Guatemala. ARCAS receives animals from seizures of illegal traffic or illicit possession of wildlife and integrates them into rehabilitation and/or reproduction programs. They go on to reinforce existing wild populations with new individuals, increasing their diversity and genetic variability by conducting monitored releases into their natural habitat. Before being released, birds are subject to serological tests and molecular diagnostic tests, including BFDV.[28] In previous results, BFDV has been detected by PCR test in blood samples of some individuals of *Ara macao* from the Rescue Center, resulting in the isolation of these individuals.

The objective of this study was to conduct a general evaluation of the main psittacine species present at the ARCAS Rescue Center, performing a statistically significant sampling to determine the presence or absence of BFDV through blood samples by real-time PCR. Since BFDV is an exotic pathogen for neotropical species and not officially recognized by the animal health offices in Guatemala due to the absence of diagnostic reports in the country in captive native or exotic birds, importing infectious material, or positive samples, was not feasible for this study. Synthetic oligonucleotide positive controls were used as material for PCR positive controls instead of confirmed positive blood samples.

Materials and Methods

Animals

All blood samples were collected at the Wildlife Rescue and Rehabilitation Center ARCAS Petén, which is a rescue center accredited by the Guatemalan National Council of Protected Areas (CONAP) and accredited by the Global Federation of Animal Sanctuary (GFAS).

Samples were collected from 117 clinically healthy birds from a total population of 309 from the following species: Scarlet macaw (*Ara macao*), Military macaw (*Ara militaris*), Great green macaw (*Ara ambiguus*), Blue-and-yellow macaw (*Ara ararauna*), Red-lored parrot (*Amazona autumnalis*), White-fronted parrot (*Amazona albifrons*), Mealy parrot

(*Amazona farinosa guatemalae*), Yellow-headed parrot (*Amazona oratrix*) and White-crowned parrot (*Pionus senilis*). Out of the entire population at the time of the study, only 5 birds presented abnormal plumage: 2 *Ara macao*, 1 *Ara ambiguus*, 1 *Ara ararauna* and 1 *Amazona farinosa*.

Birds were selected from different areas of the Rescue Center according to rehabilitation stages. Aviaries from each area have different architecture and receive different husbandry, designed and utilized for a specific purpose in the rehabilitation process. Areas for releasable animals are: Avian quarantine (Aq), Maintenance (Ma), Pre-rehabilitation (Prerehab), and Rehabilitation (Rehab). The Center also holds permanent residents, which are animals that have been deemed non-releasable and are located in a separate area known as the Biodiversity Education Center (CeBio), which has visitor access for educational purposes. Birds were sampled from aviaries in each management area according to availability (Table 1).

Table 1. Neotropical psittacine species sampled from the ARCAS Wildlife Rescue and Rehabilitation Center according to the management area from where birds were housed.

Species	Areas				
	Aq	Ma	Prerehab	CeBio	Total
Ara species					32
Ara macao	1	0	25	2	28
Ara ararauna	0	0	0	1	1
Ara militaris	0	0	0	2	2
Ara ambiguus	0	0	0	1	1
Amazona species					69
Amazona autumnalis	8	3	15	11	37
Amazona albifrons	6	10	2	0	18
Amazona farinosa	0	0	0	13	13
Amazona oratrix	0	0	0	1	1
Pionus species					16
Pionus senilis	1	14	1	0	16

Abbreviations: Aq, Avian quarantine; Ma, Maintenance; Prerehab, Pre-rehabilitation; CeBio, Biodiversity Education Center.

The sample size, according to R function pwr.chisq.test, using effect size of 0.3, power of 0.80, significance level 0.05 and 2 degree of freedom, was of 108 birds. However, the actual sample size for qPCR analysis in the study was increased since routine management allowed for more samples to be obtained. Also, the imported reagents were only temporarily available and diagnosis tests for BFDV are not commercialized regularly in Guatemala yet. Final sample size was 117, obtaining power of 0.836.

Sample collection

Blood was collected into commercial sterile 0.5 ml vials with Ethylenediaminetetraacetic acid (EDTA) by puncturing the brachial vein using sterile disposable needles and syringes. The samples were stored at 4 °C until transported to the molecular biology laboratory of the Faculty of Veterinary Medicine of the University of San Carlos of Guatemala (USAC), in Guatemala City, where qPCR test was performed. Sample collection started in June and ended in December 2019, and it was performed during routine veterinary checks between 9 a.m. and 11 a.m.

DNA extraction

DNA was extracted from 200 µl of avian blood samples with EDTA using commercial High Pure PCR Template Preparation Kit© (Roche Diagnostics, Guatemala City, Guatemala), according to the manufacturer's instructions and it was stored at -20 °C.

PCR amplification

Real-time PCR for BFDV detection was performed using Beak and Feather Disease Virus genesig® Advanced Kit designed by the company Primerdesign Ltd for the detection and quantification of BFDV according the manufacturer's handbook,[29] which contains a set of primers that targets the replication associated protein (*rep*) gene (ORF1) and amplifies a 80 nucleotide region between nucleotide positions 395 and 475. PCR amplicon context sequence is described in adherence to MIQE guidelines:[30] expected amplicon length was 125 bp and anchor nucleotide was at position 436, based on known GenBank sequence GU015023.

PCR reactions were prepared to a final volume of 20 µl, using 10 µl re-suspended oasig lyophilized 2X qPCR Mastermix with ROX dye (Primerdesign, Chandler's Ford, Hampshire, UK), 1 µl BFDV primers, 1 µl internal control primers from Beak and Feather Disease Virus genesig® Advanced Kit (Primerdesign, Chandler's Ford, Hampshire, UK), 3 µl RNase/DNase free water and 5µl of DNA template. Control exogenous DNA from Beak and Feather Disease Virus genesig® Advanced Kit (Primerdesign, Chandler's Ford, Hampshire, UK), 4 µl, was incorporate in each sample DNA extract buffer to assure PCR inhibitors were not present at a high concentration. Each run included a negative control, using 5µl RNase/DNase free water, and a positive control, using 5µl BFDV positive control template with known copy number ($2x10^5$ per µl) from Beak and Feather Disease Virus genesig® Advanced Kit (Primerdesign, Chandler's Ford, Hampshire, UK).

Real-time PCR was performed by the molecular biology laboratory of the Faculty of Veterinary Medicine FMVZ of University of San Carlos of Guatemala, using StepOnePlus Real-Time PCR thermocycler (Applied Biosystems, CA, US) under the following amplification conditions: enzyme activation at 95 °C for 2 min, denaturation at 95 °C for 10 s and amplification at 60 °C for 60 s, for 50 cycles. Samples with Ct value of ≤30 and an exponential fluorescence were scored as positive and samples with positive internal control and without exponential fluorescence were scored as negative.

Statistical analysis

Prevalence was estimated using the formula: BFDV positive birds / tested birds * 100. Prevalence and its respective C.I. were estimated for *Ara* species, *Amazona* species, *Pionus senilis* and general population. One-sided upper limit for a 95% confidence interval

(C.I.) was estimated using the formula with correction factor $p+z^*\sqrt{p^*(1-p)}/\sqrt{(N-1)^*n/(N-n)}$; as the estimated p is 0, a p = 0.50 was assumed for the largest variance, so that the one-sided upper limit is the maximum estimated adjusted by population size (N) and different sample size (n).

Results

In this study, the total BFDV (Beak and Feather Disease Virus) prevalence was 0%, for the tested species at the ARCAS Rescue Center, with general Confidence Interval (C.I.) of [0% - 6.0%]. Prevalence for *Ara* species was 0% [C.I. 0% - 11.93%], for *Amazona* species 0% [C.I. 0% - 7.90%] and for *Pionus* species 0% [C.I. 0% - 13.00%] (Table 2).

Table 2. Real-time PCR results in neotropical psittacine species tested for BFDV from the ARCAS Wildlife Rescue and Rehabilitation Center, 2020.

Species	Tested birds	BFDV positive	BFDV prevalence [one-sided 95% C.I.]
Ara species	32	0	0%, [0% - 11.93%]
Ara macao	28	0	
Ara ararauna	1	0	
Ara militaris	2	0	
Ara ambiguus	1	0	
Amazona species	69	0	0%, [0% - 7.90%]
Amazona autumnalis	37	0	
Amazona albifrons	18	0	
Amazona farinosa	13	0	
Amazona oratrix	1	0	
Pionus species	16	0	0%, [0% - 13.00%]
Pionus senilis	16	0	
TOTAL	117	0	0%, [0% - 6.00%]

Abbreviations: BFDV, Beak and Feather Disease Virus; C.I., confidence interval.

ARCAS has a sampling history of *Ara macao cyanoptera* for BFDV through PCR testing, where positive animals have been reported. The negative qPCR test results of 7 formerly positive birds that were included in this study coincide with the negative test results via conventional PCR reported in the most recent sampling (April 2019) by HealthGene Laboratory in Toronto, Canada, obtained from ARCAS database (Table 3).

Table 3. Testing results of *Ara macao cyanoptera* samples from the reproduction and reintroduction program at the ARCAS Wildlife Rescue and Rehabilitation Center, using conventional PCR (2015, 2018 and 2019) and real-time PCR (2020).

Bird ID	Year of birth	Conventional PCR 2015[a]	Conventional PCR 2018[a]	Conventional PCR 2019[a]	Real-time PCR 2020[b]
GUA 31	2008	+	+	-	-
GUA 34	2008	+	+	-	-

GUA 37	2009	+	+	-	-
GUA 39	2009	+	+	-	-
GUA 41	2010	NA	+	-	-
GUA 55	2015	NA	+	-	-
GUA 58	2016	NA	+	-	-

Abbreviations: ID, identification; PCR, polymerase chain reaction.
[a] Results from HealthGene Laboratory, Canada, from previews tests recorded in ARCAS database.
[b] Results from the molecular biology laboratory of the Faculty of Veterinary Medicine FMVZ-USAC, Guatemala, from this study.

Discussion

In this study no BFDV DNA was detected in blood samples of neotropical psittacines in captivity from the evaluated rescue and rehabilitation center in Guatemala. Those results were unexpected since recent studies in other Latin American countries reported molecular detection of BFDV in captivity in most of the species tested in this study.[3,8,9] *Amazona autumnalis* reported high prevalence of BFDV in Costa Rica in rescue centers,[8] shelters and private clinics,[9] yet in this study the virus was not detected in blood samples of this species despite the abundance of specimens.

It has been acknowledged that most of the neotropical species tested in this study (*Ara macao*, *Ara ararauna*, *Ara militaris*, *Ara ambiguus*, *Amazona autumnalis*, *Amazona albifrons*, *Amazona oratrix*), can be infected by BFDV if the conditions are adequate. [1,3,6,8,9,14,17] However, there are no positive reports of BFDV, which the authors are aware of, in *Amazona farinosa* and *Pionus senilis*.[1,8,15,17,31]

Fogell and other authors emphasize dissemination of both BFDV positive and negative screening results, since species, such as cockatiels (*Nymphicus hollandicus*), have reported susceptibility to viral infection, yet negative PCR results from different surveys suggest host natural resistance to persistent infection.[13,16] It is outside this study's scope to determine if the evaluated species are resistant to BFDV infection, however, authors consider that more information is needed about neotropical psittacines susceptibility to an established circoviral infection in captive conditions. This could help increase the recruitment of specimens for reintroduction or reproduction programs and improve the health management of rescued and confiscated birds.

The paucity of unique BFDV genotypes[3,9,27,32] and the genomes isolated in neotropical species in captivity in Central and South America suggest introduction of BFDV strains from Europe, Asia and Oceania due to the trade[3,9] of exotic pets that may act as carriers, such as cockatoos, budgerigars (*Melopsittacus undulatus*), and lovebirds (*Agapornis* species).[13,16] The exposure of illegally trafficked birds to exotic pathogens could differ according to the stage in the supply chain in which wildlife is retrieved from illegal trade. Since there are no reports of BFDV in wild neotropical flocks in the American continent, chances that smuggled nestlings could be already BFDV carriers or been exposed to carriers are low, especially if they are confiscated directly from poachers or local

intermediary sellers, or before they reach regional or international markets.[4,7,16,17] On the other hand, endemic smuggled birds sized from local or international markets, households or private collections could have higher risk of acquiring the virus[4,7,22,25,26] due to cohabitation with exotic pets.

Authors consider that the low BFDV prevalence obtained in this study could be related to the rescue center management protocols. All psittacines that arrive at the ARCAS Rescue Center undergo long quarantine periods, some up to 6 months, in which birds are allocated to individual or joint enclosures before being introduced into free-flight aviaries with conspecifics from different origins. Due to rehabilitation management, birds are separated in aviaries according to species, age, rehabilitation stage and possibilities for release. ARCAS allocates in the rehabilitation and reintroduction programs rescued wild born nestlings, while adults from household or illegal collections are separated, due to domestication and/or health problems related to captivity. More information regarding the association between illegal bird trade stages and BFDV transmission and detection in neotropical psittacines is needed in order to refine selection of candidates for reintroduction programs.

Regular cleaning and disinfection of individual and group cages in the quarantine areas, restricted contact between birds for long periods and separation of the avian population in enclosures according species, age and management stage could also helped to avoid BFDV spread by diluting viral concentration in the environment and reducing long-term exposure.[12,16]

Birds tested in this study were mostly rescued young adults and grown adults (116/117), except for 13 *Ara macao cyanoptera* that were born in the center, from parents with regular health screening that have yielded multiple negative BFDV PCR results. Although those macaws should not represent a significant biosecurity threat, BFDV was detected in blood samples in 7 macaws in previous screenings (Table 3) according to the ARCAS database. The positive birds were 2 - 8 years old during the first detection and never presented suggestive PBFD clinical signs and yielded negative PCR results in the last screenings.

Authors suspect those birds could have contracted the virus from sharing an enclosure with older macaws seized from households, but overcame the transient infection and cleared the virus from their system without developing clinical PBFD or persistent BFDV. This event would follow the documented evidence of self-limited BFDV infections and spontaneous recoveries in *Pionus maximiliani* and *Ara macao* chicks with persisting viremia that could last from several weeks to months.[21] Repeated detection of BFDV DNA in the blood samples of those young macaws previously this study could be attributed to viral DNA fragment persistence in bloodstream, as reported in other studies.[33] More information about BFDV DNA persistence in recovered neotropical psittacines is necessary since the circulating viral DNA could last longer than expected and every screening protocol and health records should consider this factor.

Health screening in neotropical species is particularly important for avian reproduction and reintroduction programs in Central and South America, since there are no reports of BFDV detection in wild birds and the introduction of a novel pathogen like this one could have

unpredictable effects due to interspecies interaction.[4,7,17] Animals rescued from illegal wildlife trade are considered less desirable for reintroduction programs than captive born individuals, since they could have been exposed to pathogens from other captive wildlife, domestic animals and even humans when traded in markets or kept as pets, which in turn may introduce exotic diseases into naïve populations.[3,7,25,26,34] Even so, birds that have high biological and genetic value for restocking wild populations, which decline annually due to poaching and habitat deterioration, should be considered for conservation efforts. The reintroduction of healthy young adults can provide wild flocks with enough individuals to sustain the effects of natural predation and poaching.[34]

Blood samples provided greater efficacy in BFDV diagnosis in neotropical species[8] and real-time PCR test is considered reliable for its detection.[10,30,35] Even so, false negative and positive results are more frequent that expected in PCR tests. Olsen and other authors report laboratories that offer veterinary services perform BFDV PCR tests with greater sensitivity (98%) than specificity (82%).[36] Therefore, it is not possible to ensure the absolute absence of BFDV in avian population of the rescue center.

However, a general prevalence of 0%, which true value is included in the interval 0% - 6.0% with 95% of confidence level, was considered acceptable. Import of biological material with BFDV for positive controls in this study was not feasible due to governmental regulations that we could not circumvent. Until standardized BDFV diagnostic test protocols are implemented in local laboratories, rescue centers in Guatemala should upgrade biosecurity, hygiene and management to avoid and/or mitigate dispersion of BFDV in captive psittacines.

Conclusion

More information about neotropical psittacines susceptibility to BFDV is needed since species like *Amazona farinosa* and *Pionus senilis* have not been found to return positive results to infection screening, which may suggest some natural resistance to an established infection.

Neotropical psittacines rescued from illegal trade can acquire BFDV. If reintroduction programs do not execute proper quarantine protocols, these birds pose a risk of introducing an exotic disease into naïve wild populations or the greater populations at the facility, setting back any conservation effort by several years, as this virus is difficult to eradicate from an aviary setting.

Long quarantine periods, regular hygiene, health screening, and separation from the general avian population in individual enclosures or batches grouped by species, age and management stage should be considered where rehabilitation and reintroduction programs are being executed.

This study resulted with a prevalence of 0%, (C.I. 95%, 0% - 6.0%). Therefore, we find no evidence of the presence of BFDV in the psittacine population at the ARCAS Wildlife Rescue Center at the time of the screening. The implementation of PCR testing for avian circovirus should be done routinely in specimens intended to be released as the BFDV

DNA persistence in bloodstream in recovered or asymptomatic neotropical psittacines could be longer than expected.

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SURVEY OF BEAK AND FEATHER DISEASE VIRUS (BFDV) IN GUATEMALAN NEOTROPICAL PSITTACINES FROM ILLEGAL WILDLIFE TRADE CONSIDERED FOR REHABILITATION AND REINTRODUCTION

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