

Independent review of ACTP's health report on exported Dominican parrots

The health report provided by Marcellus Burkle, concerning the 12 Amazon parrots removed from Dominica's Parrot Conservation and Research Centre on 17 March 2018 by ACTP, demands critical evaluation. Some of Burkle's conclusions and recommendations logically and scientifically require further investigation and interpretation by impartial scientific authorities who have no vested interest in his or ACTP's actions or recommendations. Burkle states that the 12 birds were examined and samples collected on March 21 and March 26, four days after the stress of capture from the aviary and many hours of international transport. Burkle himself states that the hematological values indicate severe stress associated with the transfer of the parrots to Germany.

Burkle reports that of the 12 parrots taken from the aviaries on Dominica, 4 *Amazona arausiaca* tested positive for specific antibodies to Psittacine Herpes Virus 1 (PHV1) by Western blot (Pd Dr Monika Rinder, Ludwig Maximilians-Universitat Munchen; 12 swabs, blood, feathers, serum were collected 289 March 2018). The 4 sero-positive birds were then tested by PCR and only one bird tested positive (T5, weak band reported). There is no indication of what sample (swab, feather or blood) was positive by PCR, a test which is extremely sensitive and for which samples must be carefully collected to prevent environmental contamination.

Burkle's requested tests, and report summary:

Avian Bornavirus- ABV (RNA and antibodies)— all negative by RT PCR and ELISA for antibodies against ABV

PBFD virus (DNA)—all negative

Avian polyoma virus (DNA)— all negative

PHV1 (antibodies)— DNA-suspect-positive for one sero-positive bird

Chlamydia (DNA)—In Dr. Burkle's summary, he references that all 12 birds were negative for chlamydial DNA but the individual health reports indicate that Chlamydia DNA was detected in a sample from one patient but the positive sample is not defined. There is no mention in the report of Chlamydia antibody titer results or the type of test that was used for serologic evaluation. Further, there was no report from an outside laboratory suggesting that these serological findings may have been an in-house test of some sort and therefore lacking independent validation.

Psittacine Herpes Virus—discussion:

According to *Undergroundweather.com*, the temperatures in Munich were low 30F/high 38F on March 17 2018. The birds would, unavoidably, have been exposed to temperatures that they never would experience in their native habitat on Dominica. Temperature-induced stress—a known trigger for PHV1 shedding— should have resulted in stronger test evidence of viral DNA (along with its inherent danger to other birds). However, only one bird tested positive by PCR—and that was a weak, perhaps questionable result. Any such suspected positive result would warrant immediate retesting, which was not done.

Burkle's interpretation of the PHV1 test results, "that all positive birds are considered to be permanently infected and shedding of the virus might occur in the future. Why were the other 8 birds not tested by PCR to detect possible shedding of PHV1? Serological diagnosis of herpes viral diseases, in general, is subjective and difficult to validate. Strong and verifiable PCR detection of viral PHV1 DNA would be necessary to conclude that the birds are actually infected with the virus.

If any of the birds were infected during the transport, it would be logical to assume that if shedding of the virus occurred, more of the birds would have become infected, and more shedding therefore would be detected. Proper veterinary and aviary management dictates that all 12 birds should be monitored serologically and by PCR for shedding on repeated exams over time before any conclusion about these birds can be made.

It is well known that viral concentration associated with indoor housing increases the risk for shedding and concentration of PHV1 virus. Housing any potentially shedding birds indoors, in proximity to, and in the same facility as the designated *ex-situ* insurance population of St Vincent's Amazons, St. Lucia's Amazons, the most significant populations of Spix's macaws in the world, as well as a significant population of Lear's Macaws, represents an unacceptable risk for all of those species and populations. If the group of Dominican Amazons represents a risk, as suggested by Burkle's interpretations, they should be returned to their home range.

Further, based upon Burkle's strongly worded conclusions following a single, weak positive PCR result, the international conservation and veterinary communities justifiably demand a second, independent opinion and verification of all results by a second laboratory. If, indeed, Burkle's conclusions are supported and these birds represent a risk to wild populations, then breeding them for the purpose of species recovery in the wild also represents an unacceptable risk. Logically, any cooperative breeding program purportedly established between Dominican officials and ACTP should be nullified. At the very minimum, the birds should be transferred to a neutral facility where they can be further studied without posing a risk to the other extremely rare parrot populations currently housed at ACTP.

Chlamydia—discussion:

Finding sero-positive birds for Chlamydia (*Chlamydophila*), all of which or one of which (depending on which part of Dr. Burkle's report is correct) were PCR-negative four days after international transport of some 10+ hours, and exposure to novel housing and cold

temperatures in transit, **is meaningful**. Burkle's conclusion— that the birds are “permanently infected, and whether a bird will start shedding the pathogen or not in the future cannot be foreseen” and, therefore, all should be held in permanent isolation— cannot be medically or scientifically justified. **This statement implies that latent infections of *Chlamydia psittaci* occurs in parrots, and specifically in these two *Amazona* species, which has not been scientifically documented.**

Excerpt from the CDC Compendium on Psittacosis (2017) defines a confirmed case of Chlamydiosis in birds - Journal of Avian Medicine and Surgery 31(3):262-282. 2017 <https://doi.org/10.1647/217-265>

A confirmed case of avian chlamydial infection is defined on the basis of at least 1 of the following:

- Isolation of *C. psittaci* from a clinical specimen.
- Identification of chlamydial DNA by use of *in situ* hybridization to detect chlamydial DNA followed by specific *C. psittaci* DNA detection using PCR-based testing of *in situ* hybridization–positive tissues or secondary *C. psittaci*–specific DNA probes in combination with characteristic pathology. The commercial antibodies used for immunohistochemistry staining cross-react with non-chlamydial epitopes and are not diagnostic.
- A fourfold or greater change in serologic titer in 2 specimens from the bird obtained at least 2 weeks apart and assayed simultaneously at the same laboratory.
- Identification of suggestive intracellular bacteria within diseased cells in smears or tissues (e.g., liver, conjunctival, spleen, respiratory secretions) stained with Gimenez or Macchiavello stain in combination with detecting *C. psittaci* DNA in the same tissue sample using a *C. psittaci*–specific PCR-based detection assay.

A suspected case of avian chlamydial infection is defined as a compatible illness and at least one of the following:

- Identification of chlamydial nucleic acid by PCR-based testing in conjunctival, choanal, or cloacal swabs, blood, or feces.
- *Chlamydiaceae* antigen (fluorescent antibody) in feces, a cloacal swab specimen, or respiratory tract or ocular exudates. **The commercial antibodies used for fluorescent antibody staining cross-react with non-chlamydial epitopes and are not diagnostic.**
- Is epidemiologically linked to a confirmed case in a human or bird.

There may be cause for concern and further investigation if one of the following occurs: 1) identification of chlamydial nucleic acid by PCR-based testing in conjunctival, choanal, or cloacal swabs, blood, or feces in a healthy-appearing bird, or 2) compatible illness with positive results from a non-standardized test or a new investigational test.

Origin of birds exported to ACTP:

All of the Dominican Amazons had been rescued after Hurricane Maria and brought to the aviary for rehabilitation, except for the female Imperial Amazon, which is 18 years old and successfully reproduced at Dominica's aviary in 2010. **If these birds are in fact infected with Psittacine Herpes Virus 1 and have been previously infected with Chlamydia, then these cases represent natural exposure to pathogens in their native habitat. Dominica is a closed system with no feral, non-native parrot populations.**

Conclusions from this review:

- The finding of antibodies to Chlamydia indicates natural infection by the bacterium in birds originating in the wild. This is a subject which could and should be investigated on the island of Dominica. ACTP funding for independent, *credentialed* university researchers would be the appropriate response to antibody-positive findings.
- There are currently no established feral populations of foreign psittacines on Dominica. No *A. imperialis* or *A. arausiaca* have EVER been taken off of the Island, exposed to other psittacines outside of Dominica and returned to the island. No other psittacines have been housed at Dominica's Parrot Conservation and Research Centre. Therefore the serological findings suggest natural infection to organisms which would logically be found in the wild population. This is an area of research that is needed before proposing any *ex-situ* population as a "safety net against extinction"—not afterwards.
- **If** in fact the 10 *A. arausiaca* and 2 *A. imperialis* which were exported to Germany on March 17, 2018 are considered permanently infected with Psittacine Herpes Virus 1 and Chlamydia, even as incorrectly defined by Dr. Burkle, then these birds represent an unacceptable risk to the St Vincent's Amazons, St Lucia's Amazons, and Spix's macaws housed at ACTP. Such significant populations of these globally endangered species exist only at ACTP. Exposing endangered parrots to indoor housing in a cold climate— known triggers for PHV1 shedding— represents an unacceptable biohazard.
- **If** in fact the sero-positive Amazons from Dominica are considered "permanently infected" and "could start shedding PMV1 virus in the future" then housing them indoors in a cold climate is reckless management.
- Housing a PHV1 "permanently infected" population of Dominican Amazons in the same facility as the majority (perhaps 95%) of the captive population of Spix's macaws, being readied for re-introduction to establish a wild population in Brazil, is reckless management of a totally irreplaceable population of a species which is extinct in the wild.
- Mixing Dominican Amazons in the same facility as Spix's macaws with a history of ABV is an unacceptable risk, especially if the conservation objective is to augment the wild populations of *A. imperialis* and *A. arausiaca*.

- Whereas Burkle's report claims that ABV has been eliminated from the captive Spix's macaw population, **conclusive, peer-reviewed evidence of this has not been provided to the scientific community.** Any such published findings should be supplied to IUCN prior to the macaws' release. **Just as disease testing of Dominican parrots should have been conducted on Dominica, this testing can be done independently in Brazil where the birds can logically be flocked prior to release, in their native climate.**

Finally, Burkle's disease interpretations and management recommendations reveal a fundamental lack of knowledge regarding the epizootiology of wild parrot populations, the life histories and ecology of Dominica's endemic parrot species and the long history of Dominica's Parrot Conservation and Research Centre. In view of the test results, Burkle's assertions are specious. Most importantly, his management recommendations are reckless considering that the exported parrots originated in the wild on Dominica, which has no non-native, feral parrot populations.

Susan Clubb, DVM, Diplomat of American Board of Veterinary Practitioners (Avian)

Patricia J. Latas, DVM

Paul R. Reillo, PhD

7 May 2018