

8.0 DISEASE ISSUES AND TESTING RECOMMENDATIONS

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8.1 Disease Risk Assessment

Introducing animals from outside into a population always carries with it some possibility of also introducing disease. If no animals of the same species are present, the level of risk is lower, being limited to the failure of the introduction effort, possible introduction of disease into related species, if present, and contamination of the environment. For a Guatemala or El Salvador effort, the plans as they are evolving generally assume new individuals will be introduced into an existing scarlet macaw population (Guatemala) or into an environment containing other wild psittacines (El Salvador). Disease risk assessment and then risk mitigation are thus of considerable importance. Risk assessment begins with compilation of as comprehensive a list of potential diseases as possible, followed by assessing the risks from each of these diseases and winnowing the comprehensive list down to a short list of diseases of real concern. The last element of risk assessment involves a risk reduction plan, including diagnostic testing. Darrel Styles, an avian veterinary virologist and aviculturist, and Bonnie Raphael, a zoo veterinarian, led this workshop discussion on Wednesday afternoon (March 12) (Figs. 8-1 and 8-2).



Figure 8-1. Veterinarians Darrel Styles (left) and Bonnie Raphael leading the discussion on avian diseases and testing needed for a macaw release program in Guatemala or El Salvador.

8.2 Problems in Using Diagnostic Tests for Screening

By way of introduction to the discussion, Darrel Styles discussed some of the problems inherent in using diagnostic tests for health screening. Two primary methods of testing include serology tests looking for a response of the animal to the organism via antibodies in blood serum and PCR (polymerase chain reaction) which identifies the actual organism [or causative agent] in blood, other tissues or secretions. In the case of RNA viruses, a more complicated reverse transcriptase PCR (RT-PCR) test must be used where the organism RNA is first converted to a DNA form.

Most diagnostic tests have performance problems when used for screening clinically healthy animals because they are designed for optimal performance in situations where the presence of disease is “enriched” Rideout, et al (2008) point out that many tests are species specific and few have been validated for wildlife species. It is often assumed that a test validated for one species – say domestic chickens – can be considered validated for the broader taxonomic group, but this is not necessarily the case. Serologic tests are especially difficult to interpret, being prone to both false positives and false negatives, particularly when not validated for the particular species being tested. Serologic tests will sometimes not be able to identify the agent, particularly if present at low levels (false negatives). Some tests cross react with related agents that may not be pathogenic, thus resulting in false positives. Serologic tests may be positive, reflecting past exposure (or cross reactivity with related agents), but the agent, disease causing or otherwise, may no longer be present in the animal.

Another problem lies in the statistics of using tests designed for disease diagnosis for the purpose of screening groups of clinically healthy animals. Whether a test performs satisfactorily differs for these two scenarios (clinically healthy versus clinically ill), and diagnostic tests perform better when the agent of interest is “enriched” in the population being studied (that is, when most members of the population are clinically ill).. When screening animals, the animals are pre-selected for absence of clinical signs, the agent is at a low level in the population, and test performance for evaluating disease status of the herd or flock declines because of the very high probability of at least one false positive.

Rideout, et al. (2008), noted that in their experience, not appreciating how common false positives can be when using diagnostic tests in wildlife species has had many seriously negative impacts on programs. These have included disrupted conservation programs, animals being removed from breeding programs, unnecessary euthanasia, and healthy animals remaining improperly suspected of a disease problem for years. They have the following four recommendations when screening clinically healthy animals for disease:

1. Choose non-species-specific tests
2. Choose tests that identify the agent
3. Expect false positives
4. Always follow-up to confirm positives
5. Use a laboratory with wildlife experience

8.3 Comprehensive List of Avian Diseases

Over the past seven years, both serology testing and some PCR testing of some birds at both Aviarios Mariana and ARCAS had been performed. The group elected to draw up its comprehensive list of avian diseases from the diseases covered by these tests, plus several others added by veterinarians in the group. The list of diseases was:

1. Polyoma
2. Psittacine Beak and Feather Disease (PBFD)
3. Psittacine Herpes or Pacheco's disease
4. Proventricular Dilatation Disease (PDD)
5. Chlamydia (*Chlamydophila psittaci*)
6. West Nile Virus
7. Avian Influenza
8. Infectious Bursal Disease (IBD)
9. Infectious Laryngotracheitis
10. Paramyxovirus 1 (PMV 1)
11. Paramyxovirus 2 (PMV 2)
12. Paramyxovirus 3 (PMV 3)
13. Infectious Bronchitis
14. Marek's Disease
15. Tuberculosis
16. Aspergillosis
17. Parasites
18. Malaria
19. Salmonella



Figure 8-2. Bonnie Raphael summarizing conclusions on the significance of various diseases in the comprehensive list.

Darrel Styles provided the group with relevant information on each of these diseases from the standpoint of a macaw captive release program, summarized in section 8.6.

8.4 Recommended Disease Screening

After considerable discussion, the group winnowed down the comprehensive list of diseases to the short list of diseases for which screening should be performed before any scarlet macaws are released into the wild in Guatemala or El Salvador (Table 8-1). For each disease, the method or methods for testing were also recommended. Dr. Styles' input here was invaluable, because as a trained veterinarian and avian virologist with extensive experience as an aviculturist he was able to supply a wealth of specialized information that probably could not be obtained anywhere else. Generally PCR (polymerase chain reaction) testing was recommended over serology, since PCR identifies the actual organism while serology looks for a response of the animal to the organism. In the case of RNA viruses, reverse transcriptase PCR (RT-PCR) must be used.

Table 8-1. Recommended disease testing for scarlet macaws for Guatemala release programs

Disease	Priority	Method	Comments
Polyoma	High	PCR	
Pacheco's disease	High	PCR	
Chlamydia	Recommended	PCR	Serology testing (DCF) may be less reliable unless the infection is recent. Participating veterinarians agreed on the value of PCR testing. Use of DCF testing may be considered
	Consider	DCF serology	
Avian influenza	Consider	RT-PCR	Consider defensive testing in case questions are raised
PMV-1 (Exotic Newcastle's disease or END)	Consider	RT-PCR or consistent serology negatives	Consider defensive testing because Newcastle's is such an important poultry disease, not because clinically healthy psittacines are likely to have it
<i>Salmonella pullorum</i>	Consider	Serology	In domestic poultry. Could infect chicks or humans or humans could transmit to other nests or birds.
<i>Salmonella typhimurium</i>	Consider	Most reliable is via culture	See above. Not as likely to be a problem as <i>S. pullorum</i>
Psittacine Beak and Feather disease (PBFD)	Recommended	PCR	Although rarely crosses over into New World populations, easily done along with other PCR tests and recommended to avoid controversy.

The PCR testing can be done with choanal and cloacal swabs. Pooled testing of up to 5 birds can be done in order to reduce costs, but individual testing would be required if any positives were detected. Costs are estimated (2008) to range from \$US 20 - \$US 50 per PCR test, depending on where the test is conducted. Additional costs would be associated with obtaining import and export permits and shipping of samples; this is discussed below. Serology tests are likely to cost \$US 10 - \$US 20 per test, or somewhat less if done at TVMDL (see below).

Successfully conducting a disease screening program with either of the two aviaries visited during the workshop (Aviarios Mariana and ARCAS) will require careful planning, and the effort should not be underestimated. The maximum time between sample collection and testing for PCR depends upon sample and preservation method and may be days, weeks, or even months. However, samples for RT-PCR must be maintained at 4° C and be processed within 24

hours. These short time frames, especially for RT-PCR, are a challenge when samples are collected in a remote location and must be sent to a distant analysis laboratory, perhaps on a different continent. Obtaining permits for both exportation of samples from Guatemala or El Salvador and importation into the country of the testing laboratory must take place well in advance of sample collection. Unfortunately, time was not available for fully discussing ways of handling these crucial details.

Among the issues that would need to be resolved include what testing laboratories to use. Some of the tests such as END could possibly be run in Guatemala or El Salvador, but no specific laboratories were identified. A list of commercial companies and organizations that could conduct tests on appropriate samples was compiled. See the company web sites for further information on what tests they can run and what types of samples are required.

- HealthGene in Toronto Canada (PCR testing of appropriate samples)
- Avian Biotech UK in Truro, United Kingdom (PCR testing of appropriate samples)
- Veterinary Molecular Diagnostics, Inc in Milford, OH (PCR testing of appropriate samples). This laboratory is one of the best exotic and avian testing laboratories in the United States and has one of the most extensive array of tests.
- Texas Veterinary Medical Diagnostics Laboratory (TVMDL) in College Station, TX The laboratory is one of the largest full-service veterinary diagnostic laboratories in the world. It is also one of the least expensive.
- Research Associates Laboratory in Dallas, TX (PCR testing of appropriate samples).
- UNAM (Universidad Nacional Autónoma de México) in Mexico – various departments have the capabilities, but a faculty member or student would need to become interested in a project

A CITES export permit from the country of origin (e.g., Guatemala or El Salvador), and the appropriate import permits from the country in which the laboratory is located will always be required when samples are shipped or otherwise transported. As of mid-2008, for a laboratory in the United States, importation permits would be needed from the US Fish and Wildlife Service (USFWS) Office of Management Authority and from the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). In addition, a USFWS Wildlife Declaration Form 3-177 must be submitted at the entry port at the time of importation. (Note: the following information was accurate as of mid-2008, but URLs and telephone numbers change, so in the future, interested parties may have to do internet searches to get this information.)

- Apply for the USFWS permit by submitting completed Form 3-200-29 “Permit for Import/Export/Re-export of Wildlife Samples and/or Biomedical Samples.” The application fee is either \$100 or \$200 depending upon whether the application is for a one-time sample or for multiple samples. (<http://www.fws.gov/forms/3-200-29.pdf>)
- Apply for the USDA permit by submitting Form VS Form 16-3. “Veterinary Permit for Importation and Transportation of Controlled Materials and Organisms and Vectors.” The application fee is \$94 and the permit is good for one year. Because of the presence of Exotic Newcastle’s Disease (END) in Guatemala and El Salvador, the samples must be sent to either a BSL-2 (Biosecurity Level-2) laboratory or else the receiving laboratory must treat the samples in such a way as to destroy END. The applicant will have to contact the intended

laboratory and describe this information in sections 9 and 10 of the form. (http://www.aphis.usda.gov/animal_health/permits/)

- Samples must enter the US through a designated port, which includes most of the major entry ports into the United States, including Atlanta, Dallas/Fort Worth, Houston, Los Angeles, Miami, New York, New Orleans and San Francisco. A list is given at http://www.fws.gov/le/ImpExp/Contact_Info_Ports.htm
- The Wildlife Declaration Form 3-177 may be obtained at the port itself or from the webpage http://www.fws.gov/le/ImpExp/Info_Importers_Exporters.htm

The Wildlife Conservation Society webpage

http://www.wcs.org/sw-high_tech_tools/wildlifehealthscience/fvp/168570/170367 also discusses permit guidelines for the United States, although many of its links were outdated as of mid-2008.

It should be apparent that considerable long term planning is needed to send samples into the United States. One recommendation is that representatives from USFWS and USDA involved in the permitting process be contacted about how long it will take to get such permits when the time approaches to apply. Generally the time will be at least several months. Telephone numbers to try are (703) 358-2104 for USFWS Office of Management Authority and (301) 734-3277 for USDA-APHIS.

Obtaining permits for importing samples into Canada or the United Kingdom/European Union is reportedly considerably easier than importing samples into the United States.

8.5 Flock Health Testing and Health Maintenance

While the group was able to come up with recommendations regarding the most important diseases for which to test if scarlet macaws are to be bred and released, time was not available to address the testing protocol including what birds should be tested (all birds in the aviary, breeding adults, or only juveniles to be released), how many times, and at what stage of life or in the breeding and release process. In many cases, screening can be done by pool testing groups of macaws or in interacting flocks (e.g., in large flights), by pooling and testing results from representative members of the flock.

Flock health maintenance issues also need consideration. Among these issues are:

1. Biosecurity and quarantine procedures
2. Routine flock health surveillance and testing
3. Routine parasite control
4. Health assurance procedures for birds for actual release

8.6 Summary of Disease Characteristics

A summary of the characteristics of the diseases on the comprehensive list considered by the group is given below. The workshop participants were extremely lucky to have Dr. Styles

present because he was able to present this summary to us from his extensive studies and experience. This information is not available from any one source or even from several sources.

8.6.1 Polyoma

Polyoma viruses are small, potentially oncogenic DNA based viruses. In birds, disease is transmitted via feather dander. In the *Ara* genus, it is typically a disease of juvenile birds before fledging. Adults can be infected but rarely die. When *Ara* genus birds are exposed prior to 12 weeks, ~100% sicken and die. Exposed after 12 weeks, they generally survive, show no clinical symptoms, and clear the virus in 60-90 days. In aviculture, the disease is typically not seen in nest boxes but rather in nurseries. Infection rate in nurseries approaches 100%. The disease is not medically treatable but is controllable in aviaries through proper management. In the wild it would be expected to cause loss of production in individual nests, but not to be spread from one nest site to another. The risk in Guatemala would be due to exposure to birds in the pet trade, but for birds being introduced from the two captive collections examined, the risk is considered low. Poultry viruses cross react in the serology test, so false positives are possible. Testing should be done via PCR

8.6.2 Psittacine Beak and Feather Disease (PBFD)

The disease is caused by a circovirus. The origin is not known, and the host species are unknown. It may be of African origin. Lovebirds (*Agapornis*) and budgerigars (*Melopsittacus*) can be carriers. Guatemala receives shipments of lovebirds from Cuba for the pet trade, so the disease could potentially enter the country via such shipments. Wild parrots have been infected, with the most serious (present) impact seen in cockatoos and lorries in Australia. The disease acts through immunosuppression. It generally affects young birds, but can also infect adults. The disease is highly unlikely to pass into New World psittacines, as they typically clear the virus quickly. The infection rate is low, morbidity is low and fatality rate is low. An experimental vaccine exists for prevention. For birds being introduced from the two captive collections examined, the risk is considered low. In a source population exposed to other than New World psittacines, the risk should be considered moderate. Testing should be done via PCR.

8.6.3 Psittacine Herpes or Pacheco's disease

The disease was first described in the 1930's in Brazil by a Dr. Pacheco; hence the name. New World psittacines seem to be more susceptible than Old World parrots from Australasia and Africa. There is one documented case of a Keel-billed Toucan succumbing to the disease. Some species of conures are thought to carry the virus asymptotically in captivity and the length of time they shed the virus is unknown. There may be other hosts. The disease has never been detected in the wild by PCR, although some serological positives from Costa Rican and Peruvian psittacines have been reported.

The disease infects both *Ara* and *Amazona* genera, and the outcome depends on which of 4 possible strains are involved:

- Strain 4 will kill *Ara* species but not *Amazona* species.

- Strain 3 usually does not kill *Ara* species but causes persistent infection. Strain 3 kills *Amazona* species.
- Strains 1 and 2 are rare in the New World

Birds with papillomas are Pacheco's positive and carrier of one or more of the strains. The infection rate in outdoor aviaries can be moderate, but the disease can be controlled by biosecurity. The infection rate can approach 100% in indoor aviaries. The virus not thought to pass into the egg, so persistently infected macaws may be used for breeding if eggs are pulled and fostered or artificially incubated. This disease causes acute mortality so it is not likely to be introduced from captive collections, and there is a low risk of obtaining it in the wild unless papilloma positive macaws carrying the virus are released.

There is no practical treatment. There has been some success in captive parrots treating with the antiviral drug acyclovir followed by supportive therapy, and acyclovir can prevent infection. Testing should be done via PCR.

8.6.4 Proventricular Dilatation Disease (PDD)

At the time of this workshop, PDD is a histopathological diagnosis, not a disease diagnosis because the causative agent or agents is/are unknown. A bornavirus has been implicated; or the disease may result from multiple interacting factors. It is an area of active research as of mid-2008 and considerably more is likely to be understood about the disease in the next few years.

The disease is known to occur in New World psittacines, especially macaws, but it also afflicts multiple species including toucans, free-ranging Canada geese, spoonbills and weaver finches as well as Old World psittacines from Asia, Australia, and Africa. It is an autoimmune disease, with two manifestations: gastrointestinal and neurological. Mortality approaches 100%. Transmission routes are unproven. No tests are currently available and there is no treatment except supportive therapy. Since it cannot be tested for and already exists in New World birds, the only way to deal with it is not to release any birds with symptoms or birds that have been around symptomatic birds. This recommendation is likely to change in the future as tests and possibly immunization are likely to emerge.

8.6.5 Chlamydia / Chlamydophila (*Chlamydophila psittaci*)

Chlamydophila psittaci is a bacterial organism, but it can't be grown in agar, it must be grown in cells. The organism can infect people, where it causes severe flu-like symptoms and fevers because the organism affects the temperature regulatory system. Infection can cause long term health problems. There is a minimal infection risk from wild psittacines because the disease is not maintained in wild bird populations as those that are sick die or are predated. However, a significant percentage of urban pigeons in Guatemala are likely to be infected. Cockatiels and other carriers may shed asymptotically for at least a year. Infection occurs via the oral-nasal route. The disease can cause reproductive problems in breeding birds. The infection rate in open aviaries is low and the infection rate is density dependent. The disease can be treated medically with doxycycline and related drugs. Transmission in the wild is likely to be low, and the likelihood of transmission from captive collections is moderate. PCR should be used if testing is

performed. Serologic testing (DCF) may be useful for detection of previous infection and could be considered as an ancillary chlamydia diagnostic test.

8.6.6 West Nile Virus

WNV is a member of the family of RNA arboviruses and originated in Africa. Some bird species can be carriers. Corvids, raptors, and flamingos are very susceptible, with high viremia leading to liver disease and rapid mortality. WNV can infect many species of birds but only some become sick. The disease affects all life stages. It has already been documented in Central and South America (as of 2008). The disease is usually transmitted by mosquitoes, except in some flocking birds via lateral transmission. Death rates seem lower where mosquitoes are found year-round—native arboviruses may provide some cross-protection. Psittacines can show clinical signs but can't transmit the disease because the viremic phase does not reach the threshold of infection for mosquitoes. A macaw experimentally infected showed some symptoms in 10-14 days. Because it is an RNA virus, it would require testing via RT-PCR, something difficult to do in most developing countries. Testing for WNV is not considered necessary for aviaries or pre-release health screening in Guatemala or El Salvador.

8.6.7 Avian Influenza

Avian influenza is of worldwide occurrence. The low pathogenic version is a natural infection of juvenile waterfowl and shorebirds. If the virus passes through chickens it can mutate to the high pathogenic form. Psittacines can be experimentally infected with the high pathogenic form. Adult psittacines in the wild probably don't get AI, but in an aviary situation, close to chickens, ducks and guinea fowl, psittacines could become infected. Testing could be done to head off any questions by authorities, but since it is an RNA virus, testing would need to be done by RT-PCR or an antigen strip test.

8.6.8. Infectious Bursal Disease (IBD)

Not a disease of psittacines so of no concern and no testing needed.

8.6.9 Infectious Laryngotracheitis

Very limited occurrence in psittacines; not important, no testing needed.

8.6.10 Paramyxovirus 1 (PMV 1)

PMV I is Newcastle's disease, an economically important poultry disease. Psittacines can get Newcastle's, where the infection rate is high and it causes high morbidity and high mortality within 5-7 days. There is a very low likelihood of this disease entering a wild population from birds in the aviaries visited. Infection from domestic chickens or people carrying it on clothing, footwear, etc., is a more likely route of infection of wild psittacines. Because this disease resides in poultry, exposure is more difficult to control and this disease may have a likelihood of being introduced into the wild, even in the absence of releases of captive birds. Unfortunately, once in a population it could be devastating because it causes acute and high mortality rates. It is an

RNA virus; so requires RT-PCR test for definitive diagnosis. Serology positives or negatives are probably indicative and because it is an important poultry disease, defensive serology testing of clinically healthy macaws in a release program could be advisable. Any serological positives should be retested.

8.6.11 Paramyxovirus 2 (PMV 2)

A poultry disease only. Of no concern for psittacines and no testing needed.

8.6.12. Paramyxovirus 3 (PMV 3)

A disease of turkeys. It has been implicated / associated with proventricular dilatation disease (PDD), but the relationship is not proven. The virus can infect psittacines and causes CNS symptoms until recovery. Low mortality. An RNA virus; so requires RT-PCR testing. Serology positives or negatives are probably indicative of present or past infection.

8.6.13 Infectious Bronchitis

Not a disease of psittacines and no testing needed.

8.6.14 Marek's Disease

Not a disease of psittacines and no testing needed

8.6.15 Tuberculosis

In psittacines, infection is by *Mycobacterium avium* and *M. genavense*. The disease is not likely to be a problem in Guatemala or El Salvador, but *Brotogeris* species in captivity have sometimes been found to be infected. Very rarely people have given TB to birds. The disease has low morbidity, low mortality, and infection is for life. There are no good tests. Serology doesn't work; and PCR is not likely to be useful because birds do not shed sufficient organisms in their secretions and feces. Only PCR from selected tissues on necropsy can detect infection.

8.6.16 Aspergillosis

Aspergillus is a genus of about 200 fungal species. It is ubiquitous in the environment, commonly occurring on starchy foods such as corn (especially if grown under drought stress) as well as on peanuts. Infection can cause respiratory disease, but the disease is rarely a problem in adult birds unless they under stress or have compromised immune system. *Aspergillus* also produces mycotoxins, with an unknown effect on birds. No testing is required.

8.6.17 Parasites

- Ectoparasites: the worst are parasitic flies. Also mites, lice, and ticks. Control with permethrum (permethrin) or carbaryl (Sevin)

- All captive psittacines with outside access should be periodically wormed. Control with pyrantel pamoate, fenbendazole, or ivermectin
- Coccidia probably not important in psittacines, although unconfirmed reports exist
- Tapeworms not common in Central or South America in psittacines. Control with praziquantel (Droncit) or epsiprantel (Cestex).

8.6.18 Malaria

Actual malaria is very rare in psittacines. The blood parasite hemoproteus is very common in macaws and can't easily be differentiated from malaria. Both types of protozoa are already in the environment and are natural commensal infections of many birds. Testing is not needed for clinically healthy birds.

8.6.19 Salmonella

Rodents and other vermin can carry the organism. Most important is probably *Salmonella pullorum* typhoid. The disease can cause mortality in chicks, and reproductive failure is possible. There is a moderate risk to wild populations from captive collections, from humans, or from domestic poultry. Transmission from nest to nest by humans handling chicks or nests is possible. Testing for detection of the disease or carriers is by serology and cloacal culture. Any poultry lab should be able to do testing.

LITERATURE CITED

Rideout, B., Morris, P., Lieberman, A. 2008. Disease Risk Assessment for Reintroductions. First International Wildlife Reintroduction Conference, April 15-16, 2008, Lincoln Park Zoo, Chicago IL.

