AVIAN CHLAMYDIOSIS SCREENING OF FREE-RANGING PSITTACIDS AT SUPPLEMENTARY FEEDING SITES IN AUSTRALIA

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INTRODUCTION

Crimson Rosellas (*Platycercus elegans)* and Australian King-Parrots (*Alisterus scapularis)* are two free-ranging psittacids that are commonly fed by the public in Australia as part of wildlife tourism and backyard bird feeding activities. These parrots are considered to be naturally susceptible to Avian Chlamydiosis caused by *Chlamydophila psittaci.* Concerns over the potential for the transmission of this zoonotic disease at public feeding sites, and the subsequent public health aspect, resulted in an initial survey for the presence of Avian Chlamydiosis in parrots at two major feeding sites. These surveys, testing antibody prevalence utilising a serological test (Immunocomb®) and antigen prevalence via Real Time PCR, were undertaken at sites in Queensland (*n*=21) and Victoria (*n*=33) in February and July 2008 respectively. Whilst results varied between the sites, they provide clear justification for further investigation including a formal risk assessment — relative to wildlife and human health issues, in order to guide appropriate preventative management strategies being reviewed and implemented. (Risk assessment and management implications are being reviewed in conjunction with an overarching research project.)

Implications for aviary birds and Avian Veterinary Practitioners are sought in open discussion.

MATERIALS AND METHODS

Data collection

Blood samples and fresh faecal swabs were collected in 2008 from the primary target species, free-ranging Crimson Rosellas and Australian King-Parrots (and secondary target species Sulphur-crested Cockatoos *Cacatua galerita* due to their close association with the feeding site in Victoria). Two study locations were preselected, each having a long history of public hand feeding of wild birds. Location 1 in Queensland is located in South East Queensland. Location 2 (with two catching localities[1] VG1 and VS2 approximately 2.5 kilometers apart) is in Victoria. The respective sample groups consisted of *n*=21-14 Crimson Rosellas and 7 Australian King Parrots in Queensland, and VG1 *n*=21- 11 Crimson Rosellas, 7 Australian King-Parrots and 3 Sulphur-crested Cockatoos, VS2 *n*=13 11 Crimson Rosellas and 2 Australian King-Parrots; combined *n*=33: Environmental samples were also collected at the designated feeding site in Victoria.

[1] Observations indicate birds from VS2 also attend VG1.

Individuals were caught using hand nets and baited netting boxes whilst feeding at the hand or floor. The birds are considered to be habituated making these capture methods highly reliable. The sampling strategy was partially randomized by pre-selecting a section (north, south, east, west) within the total area of each locality and position (hand or foot). If a bird was not at this point, the next closest was selected.

Catching efforts were undertaken by the principle field investigator and registered assistants experienced with bird handling and catching. Generally two birds were caught at a time, placed in a holding cage and covered to prevent capture myopathy.

Data collection and sampling protocol for each bird were conducted at the field laboratory (located within 2 minutes walking distance of the capture site in Queensland and 3 minutes driving distance of the capture sites in Victoria). This involved conducting a physical examination noting any abnormalities as well as age approximation and sex classification using plumage indicators (sex will be confirmed via genetic sexing), recording body weight and morphometrics (bent flattened wing length WL, culmen CL and maximum tarsus TZ using techniques described by Gosler, 2005), and pectoral profiling to assess body condition (Fowler 2008); colour marking under the Australian Bird and Bat Banding Scheme (A.B.B.B.S) using a 13 Schema, by a licensed bander 2802; and a conjunctiva, choanal and cloacal swab for DNA analysis using real time polymerase chain reaction RT PCR testing (Queensland samples).

Sample collection also involved venipuncture of the right jugular vein using a 3cc/mL syringe with a 25G 5/8 needle to collect a blood sample, 1.3ml from the Crimson Rosella and 1.5ml from the Australian King-Parrot (less than 1% of the bird's body weight). One spot of blood was placed on the ImmunoComb® card directly from the non-heparinised needle, air dried, then placed in a clean envelope and a snap lock bag for refrigeration until testing (generally conducted within 48 hours after collection following the standard protocol for the use of the kit); a heparinsed microhematocrit tube was filled and centrifuged within 10 minutes of collection and read on a Phillips Drucker Micro Hematocrit Reader to obtain the packed cell volume; a whole blood smear was made directly from the needle and dried within 2 minutes of blood collection. The slides were sent to the Biochemistries Laboratory, School of Veterinary Science, University of Queensland, St Lucia, for staining using a Wright-Giemsa stain, for microscopic review by the principle field investigator to obtain an estimated white blood cell count and differential The remaining portion of the blood was centrifuged on site and the plasma sent to the aforementioned lab for biochemistry analysis (Albumin g/L, Glucose mmol/L, Globulin g/L, ALP u/L, LDH u/L, Amylase u/L Lipase u/L, AST u/L, Protein g/L, Calcium mmol/L, Trig mmol/L, Cholesterol mmol/L and Uric Acid umol/L). Additional portions have been stored for further RT PCR analysis (by University of Georgia, Avian Testing Laboratory - results pending).

A fresh faecal sample was collected prior to the physical examination or on completion by placing the individual into a clean holding cage (disinfected and dried thoroughly between each bird) with a numbered paper liner. A sterile cotton tipped swab was passed through each sample, labeled, bagged and refrigerated prior to forwarding by courier for laboratory testing (Victoria). Birds were either returned to the darkened holding cage or a clean calico bag for 3-5 minutes, then checked for appropriate responsive behavior prior to being transported for release nearby (within 50 metres) of the capture point.

Environmental swabs were taken at VG1 from the floor and rail within the designated feeding area and a third swab was taken from a picnic table located in the area adjacent to the feeding site. A clean cotton swab was run over the surface of a randomly selected section for approximately 2

minutes, placed into an individual clean snap lock bag with both the sample and bag labeled. These samples were forwarded with the fresh faecal swabs for processing. (Queensland environmental samples are pending results.)

All work was undertaken with approval of the University of Queensland Animal Ethics Committee - Wildlife #SVS/347/07/VAR/SF, and the relevant State authority - Department of Sustainability and Environment # 10004543 and Queensland Parks and Wildlife WISP04635807 and A.B.B.B.S permits.

Given concerns over zoonotic diseases, all field assistants were required to wear personal protective gear, including a surgical mask, gloves and laboratory coats for those in close contact with the birds. Personnel were also asked to observe strict risk management protocols including personal hygiene requirements and washing field clothes separately.

All sample management protocols of relevant laboratories were followed.

Sample analysis

Serological testing was undertaken using ImmunoComb®, an avian *Chlamydophila psittaci* antibody test kit based on the immunoassay principle designed to determine IgG antibody titers in avian blood. All blood spot samples were developed strictly according to the prescribed protocol, noting the critical control points. The results of the comb were read by the principle field investigator and confirmed against a review provided by a second person.

Molecular testing using RT PCR was also undertaken. Two separate RT PCR tests were conducted on the Victorian samples. The first targets the OMP1 Chlamydia/Chlamydophila gene, the second test targets the 16S rRNA Chlamydia/Chlamydophila gene (Refer Dr Susan Bibbey, Veterinarian, Technical Services, Scolexia Animal and Avian Health Consultancy, 8/19 Norwood Crescent, Moonee Ponds 3039). The Queensland samples were forwarded to Dr James Branley, Infectious Diseases Physician and Microbiologist, Head - Department of Microbiology, Nepean and Blue Mountains Pathology Service. These tests are considered to have a high degree of specificity and sensitivity, but neither test currently has these parameters quantified or validated.

The American National Association of State Public Health Veterinarians (2008) determines that these tests are capable of determining probable cases of Avian Chlamydiosis.

Recommended diagnostic tests recognized as 'gold standard' such as isolation via culture, to *confirm* Avian Chlamydiosis (National Association of State Public Health Veterinarians 2008) were beyond the resources available to this investigation. However, it has been argued that molecular techniques may offer greater sensitivity and there is a need for review of standards given developments in this field (Phalen, 2001).

RESULTS

These results are reported as a subset of the data obtained for an overarching PhD research project.

Serological Results

Serological results are indicative of a bird having been exposed to the pathogen and having mounted an immune response. The test results indicate a low or high positive if antibodies to *Clamydophila psittaci* have been detected. An accurate, positive result is proof that the individual has been infected

(Phalen, 2001) at some point in time, although the infection may not be current. Negative results can be indicative of a bird that has not been exposed or a bird that is in a prepatent period (Phalen, 2001). Whilst these points should be considered when interpreting these results, serologic assays have been commonly used for flock screening and where there are a significant number of positives, this is generally considered sufficient proof that the infectious agent is/was present and management action should be considered accordingly (Phalen, 2001).

ImmunoComb® results for Queensland

Localities	Negative	Positive - Low	Positive - High	Total
1	9	10	2	21

Species review for positives
Positive Low – 6 Crimson Rosellas and 4 Australian King Parrots
Positive High – 2 Australian King Parrots

ImmunoComb® results for Victoria

Localities	Negative	Positive – Low	Positive - High	Total
VG1.	7	6	7	20
VS2.	1	5	7	13
Total	8	11	14	33

Species review for positives
Positive Low – 9 Crimson Rosellas, 2 Australian King-Parrots
Positive High – 10 Crimson Rosellas, 0 Australian King-Parrots, 3 Sulphur-crested Cockatoo

Low positives are identified with an ImmunoComb® reading of 1 or 2, and high positives with ImmunoComb® reading of 3 through to 6). The sensitivity of this test has been reported to be 95% and the specificity at 85% (Whittle, 2008), with some variability among species tested (Biogal, 2005).

Polymerase Chain Reaction Results

Polymerase Chain Reaction detects the DNA specific to the disease agent. There are varying sensitivities and specificities to these tests as previously noted.

OMP1 PCR *C.psittaci* results for Queensland

Localities	Negative	Positive	Total
1	21	0	21

OMP1 PCR *C.psittaci* results for Victoria

Localities	Negative	Positive	Total
VG1.	20	0	20
VS2.	13	0	13
Total	33	0	33

Sensitivity and specificity has not yet been prescribed for this method (Bibby, 2008).

16S rRNA PCR *C.psittaci* results for Victoria

Localities	Negative	Positive	Total
VG1.	19	1	20
VS2.	13	0	13
Total	32	1	33

Species review for positives – 1 Crimson Rosella = Adult (caught at the hand within the designated feeding area at VG1).

Positive bird – likely female (waiting on genetic testing results); bird had dirty vent area, a high antibody titre on the ImmunoComb® and was in reasonable body condition.

Physical Examination

Whilst a diagnosis of avian chlamydiosis using serological testing generally requires serial samplings, it is suggested that a presumptive diagnosis can be made for a flock of wild birds if a high antibody titre is recorded for the majority of birds, with clinical signs typical of the disease present (Andersen, 2007).

Birds (with variation within the group) in the serologically positive group displayed various obvious clinical signs associated with Avian Chlamydiosis including dyspnoena, diarrhoea (Vanrompay, 2000), sparse dark green faecal material, emaciation and dehydration with packed cell volume greater than 55% (Andersen & Franson 2007). The last two signs are associated with more severely affected birds (Andersen & Franson 2007). In addition several birds had nasal discharge, many displayed poor feather condition and/or feather loss and dirty vent areas. It should be noted that although these signs have been associated with Avian Chlamydiosis they are also nonspecific and affected birds can show no or minimal overt signs (Andersen & Franson 2007).

Environmental testing for *Chlamydophila psittaci*

Environmental testing results for the swabs made at VG1 are provided.

Sample	OMP1 PCR *C.psittaci*	16S rRNA PCR *C.psittaci*
E1. Floor	Negative	Negative
E2. Rail	Negative	**Positive**
E3. Table	Negative	Negative

Sensitivity and specificity has not been prescribed (Bibby, 2008), although an increase in both specificity and sensitivity as compared to the OMP1 PCR *C.psittaci* test is expected. Further review awaits publication of the method (Bibby, 2008).

An epidemiological review is pending subject to finalisation of the entire sample sets' result.

DISCUSSION

Given positive results, general concerns held by wildlife management agencies over the activity of wild bird feeding and its high participation rates in Australia {there are 5 known high intensity feeding sites in Australia and backyard feeding rates are reported between 30% - 60% of householders in rural

and suburban areas (ABC 2008, Ishigame & Baxter 2007, Howard & Jones 2004)} the aim of presenting this paper is to generate discussion over considerations of the Avian Practitioner relative to backyard and wildlife tourism bird feeding, relative to:

1. Submission and treatment of wild birds associated with wild bird feeding activities,
2. The activity itself, and
3. Implications for aviary and pet birds.

By way of introduction to the discussion a general review of the results and the current situation is provided:

- Given an informal review (formal review pending results of overarching PhD project) of the results it is apparent that variation in the prevalence rates of the disease exist between the two sites, with the Victorian site being considered to be in a higher risk category both relative to wildlife and human health. (Both situations have been reviewed by the state Biosecurity Departments).

- Variations between the locations include spatial characteristics of the feeding sites, abundance and diversity of birds being fed, intensity and duration of public feeding within the area available, environmental management practices as well as extensive geographical differences including habitat fragmentation and weather conditions.

- The Queensland location is surrounded by extensive rainforest whereas the Victorian area is much smaller in size — with urban development dispersed throughout the park, backyard bird feeding is a common practice and incidents of Psittacosis have been reported. In addition, one length of the outer boundary of the park abuts the city of Melbourne.

- All species of birds are known to be seasonally migratory, moving to lower lying areas to feed during winter months (Forshaw 2002). Recently, one of the marked birds in Queensland was found over 100klms away in a suburban area. It was found injured and placed in a family pet birds' cage. The person who had taken the bird into care advised that they had transferred it to a box to return to the investigator, and they had already purchased a pet bird that had been placed in the cage where the wild bird had been housed.

- On dealing with a supplier of rings, the proprietor advised that when he required breeding birds he would travel to one of the feeding sites to obtain individuals.

- The vast majority of the birds were caught at the hand, offering a small amount of seed in the same way visitors participate in the bird feeding activity. This is an extremely popular activity with current visitor numbers to the site in Victoria estimated to be in excess of 500 000 and Queensland, in excess of 300 000. Birds have entered peoples' vehicles and many visitors have asked if they can take one of the birds home.

Also of concern, many of the participants in the activity are overseas tourists potentially from areas where the general public would not normally be associated with wild birds, increasing the significance of public health risk as General Practitioners overseas may not be aware of the presentation of this

disease. Psittacosis commonly presents as a flu like virus in humans but requires treatment with specific anti-bacterial medication, deaths from the disease have been recorded in Australia (Telfer *et al* 2005). As such, risk management at feeding sites and in backyard feeding locations seems a must.

Implications for Avian Veterinarians Open for Discussion

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