Antibody Testing for *Chlamydia psittaci* using a rapid ELISA-KIT

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Abstract. The Immunocomb (IC) ELISA test and immunofluorescence (IF) microscopy were compared on their ability to diagnose *Chlamydia psittaci* infection. Birds with and without clinical signs suggestive of chlamydiosis were tested. Although both test methods can detect infection by *C. psittaci*, the IC test is more convenient and provides the benefit of "on site" laboratory testing.

Introduction

The Immunocomb test kit for *Chlamydia psittaci* antibody determination in psittacine birds was developed in 1993.[1] The kit compared favorably with two other commercial tests .[2] This paper presents further data demonstrating the sensitivity and specificity of the Immunocomb (IC) method, compared with detection of fecal *Chlamydia psittaci* antigen via the direct immunofluorescence (IF) method.

Material and Methods

Cloacal swabs and blood samples were obtained from 82 psittacine birds. Forty-eight of 82 birds (58.5%) were suspected to be infected with *C. psittaci* based on clinical symptoms which included oculo or nasal discharges, sinusitis and air sacculitis.

The cloacal swabs were smeared on glass slides and fixed with 5% formalin. All slides were submitted to The Poultry & Bird Health Laboratory (which is the National Chlamydia Reference Laboratory at The Kimron Veterinary Institute, Bet Dagan, Israel), for testing via microscopic immunofluorescence.

The blood samples were collected by clipping the bird's toe nail and allowing drops of blood to saturate the pre-punched filter paper disks that are provided in the Immunocomb kit. The samples were air-dried and submitted to Biogal Galed Labs for Immunocomb testing. Tests were performed in the Chlamydia Research & Development Laboratory.

The Immunocomb tests for antibodies to *C. psittaci* were performed with commercial kits. Development of the tests and reading of results were carried out according to the enclosed instruction manual. Test results that showed a color reaction that exceeded the negative control were considered positive. A result that was comparative to the negative control (usually no color or a trace shadow) was considered negative.

Test results from these 48 birds are listed in Table 1. The remaining 34 samples (shown in Table 2) were collected randomly from birds that had no clinically apparent manifestations of chlamydial infection. Table 3 is a summation of Tables 1 & 2 and, therefore, includes the test results from all 82 birds. The tables were compiled after the test results from each laboratory were reported independently. As such, these data represent the findings of a double-blind study.

Table 1 . Immunocomb and immunofluorescence results in birds with clinical symptoms suggestive of chlamydiosis (n = 48)

Immunocomb	Immunofluorescence		Totals
	+	**-**	
+	35	2	37
-	2	9	11
Totals	37	11	48

Sensitivity = 35/37 x 100% = 94.6% (Confidence Limits [95%] = 86.0% to 100.0%)

Specificity = 9/11 x 100% = 81.1% (Confidence Limits [95%] = 54.5% to 100.0%)

Table 2. Immunocomb and immunofluorescence results in birds with no clinical symptoms of chlamydiosis (n = 34)

Immunocomb	Immunofluorescence		Totals
	+	-	
+	3	5	8
-	0	26	26
Totals	3	31	34
Sensitivity = 4/4 x 100% = 100.0% (Confidence Limits [95%] = Unable to calculate)			
Specificity = 26/30 x 100% = 86.7% (Confidence Limits [97%] = 72.8% to 100.0%)			

Table 3. Cumulative results of Immunocomb and immunofluorescence tests

Immunocomb	Immunofluorescence		Totals
	+	-	
+	38	7	45
-	2	35	37
Totals	40	42	82
Sensitivity = 38/40 x 100% = 95% 2 seronegative shedders (5% "False" Negative)			
Specificity = 35/42 x 100% = 83% 7 seropositive non-shedders (17% "False" Positive)			
Positive predictive = 38(38+7) x 100% = 84%			
Negative predictive = 35/(35 + 2) x 100% = 95%			

Conclusions and Discussion

In this study, the Immunocomb (IC) test demonstrated reliability when compared to the immunofluorescence (IF) test for identification of chlamydiosis-infected birds. The validity of sensitivity and specificity calculations must be evaluated in light of the fact that the IC and the IF techniques are testing different pathophysiologic parameters of infection which are not always present at the same time.

A false-positive IC result may be explained in an infected bird that was not shedding *C. psittaci* at the time of the test. Alternatively, a false-negative IC result would be expected in a bird during the acute stage of infection before a detectable antibody titer has been produced or in the terminal stage. Therefore, a third confirmatory test would be needed to clarify the discrepancies of test results. In addition to acceptable positive and negative prediction values, the IC test offers a decisive advantage over the IF test as a ready-to-use, standardized kit that can be performed by the veterinarian or technician outside of a conventional laboratory. The possibility of achieving rapid results in a veterinary clinic, quarantine station, or commercial aviary enhances the diagnosis of psittacosis (chlamydiosis) and facilitates the timely treatment and control of this important zoonotic disease.

The identification of infected birds is important in the control of avian chlamydiosis. The detection of chlamydial antigen in the feces via direct IF is suitable as a diagnostic screening tool from the standpoint of sensitivity, specificity, and cost of the test. The reliability of this method is reduced, however, because shedding of chlamydial elementary bodies is inconsistent. Therefore, serial testing may be required to confirm infection. A second limitation of the IF antigen test is the

requirement that it be performed in an appropriate laboratory, which obviously limits its use in the field.

Diagnostic serology to test for chlamydial antibody has been suggested as a complimentary or alternative test to fecal antigen detection.[3-5] The Immunocomb antibody test kit provides such an alternative. The test has the further benefit of being able to identify infected birds that may have temporarily ceased shedding organisms because of inadequate antibiotic treatment prior to testing. These cases of "masked infection" are of concern to importers and to quarantine stations. Since its introduction into Israel and Europe in 1995, the IC test kit is receiving increasing use by veterinarians to screen flocks for chlamydiosis and to diagnose the disease in individual birds. The kit is appropriate for testing in a wide range of psittacine and non-psittacine species (please see addendum, Table 4).

Table 4. Immunocomb detection of anti-chlamydia antibodies in various avian species (addendum 04/24/98)

Psittacine Birds	Colour (*) Intensity	Non-psittacine Birds	Colour Intensity
African Grey Parrot	+++	Turkey	+++
Macaw (*Ara* sp.)	+++	Peacock	+++
Timneh Grey Parrot	+++	Pheasant	+++
Conure	+++	Guinea Fowl	+++
Amazon Parrot	+++	Ostrich	++
Cockatoo	+++	Quail	++
Rosella	+++	Mynah Bird	++
Lovebird	++	Owl (Uhu)	++
Parakeet (Budgerigar)	++	Black Kite	++
Princess Parakeet	++	Vulture	++
Lorikeet	++	Toucan	++
Cockatiel	++	Pigeon	+
		Pelican	+
		Swan	+
		Eagle	+
		Starling	+

* = Colour intensity relative to that for the African Grey Parrot

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