Diagnosis of Psittacosis in Parakeets

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THE RECENT remarkable increase in the popularity of parakeets (budgerigars) as household pets poses a question of whether this trend has been accompanied by a rise in the incidence of psittacosis. With increasing awareness of this disease, there has undoubtedly been a tendency to diagnose and report cases which in other times might not have been identified. Nevertheless, there appears to have been a real rise in the attack rate of psittacosis (*1*, *2*). This emphasizes the need for a rapid and simple diagnostic test for psittacosis that can be used by local laboratories.

The clinical resemblance of psittacosis to other forms of pneumonitis and influenza-like illnesses makes confirmation of the diagnosis by laboratory methods almost mandatory. Early antibiotic therapy, so often employed today, makes impossible the diagnosis of psittacosis by isolation of the virus from suspected human cases. Such therapy may also suppress or delay antibody development, leaving the physician without any direct laboratory support for his clinical diagnosis. Quite often the pet parakeet or other psittacine contact may be the only supportive evidence for such diagnosis.

Psittacosis virus may cause an apparent or inapparent infection in birds, but even in the overt disease the symptoms are not pathognomonic. Moreover, apparently healthy birds frequently shed psittacosis virus. Physical examination of the bird is insufficient for a correct diagnosis in sick birds and entirely inadequate for the detection of silent infections. The isolation of the virus from infected birds offers the most accurate diagnostic method, but it entails sacrificing of the birds to obtain tissues, a procedure often objected to by an owner for sentimental reasons and by breeders or dealers for economic reasons. Therefore, a less expensive test was sought, one which would reliably detect infection in live birds.

Serologic tests, particularly complement fixation tests, have proved to be of diagnostic value with human serums (*3*). The complement fixation test has also been found to be useful in tests with pigeon and parrot serums (*4*, *5*). The serums of domestic fowl, while not reacting in the usual complement fixation test, will yield definitive results when tested by the indirect complement fixation test (*6*, *7*). The serums of sea birds infected with psittacosis virus also have been shown to react in the direct complement fixation test (*8*).

It appeared desirable, therefore, to compare

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the results of the complement fixation test on serums of parakeets bled during life with results of virus isolation attempts from their droppings and from their tissues when they were subsequently sacrificed and to investigate the possibilities of practical application of serologic techniques for the detection of infected flocks.

Materials and Methods

The parakeets in this preliminary study were obtained from 19 different owners of small "backyard" aviaries in the vicinity of Albany, Ga., except for one flock which was selected from a larger group confiscated by the State of New Jersey.

Bleeding of Parakeets

Serum was obtained from the parakeets by bleeding from the jugular vein, using a 25-gauge, ½-inch hypodermic needle attached to a 1.0-ml. tuberculin syringe which was previously wetted with physiological saline solution. The parakeets were anesthetized with ether before bleeding. One ml. of blood was withdrawn and the serum separated from the clot by centrifugation. The birds withstood the removal of 1.0 ml. of blood quite well. In fact only one bird of this series died following the bleeding, and this death was apparently caused by excessive anesthesia.

Antigen

Two psittacosis antigens were used. The first antigen was prepared from allantoic fluids of chick embryos infected with psittacosis virus recently isolated from a parakeet. The embryos were inoculated intra-allantoically with 10^{-1} or 10^{-2} dilutions of virus on the eighth day of incubation and the fluids were harvested 5 to 6 days later. These were centrifuged at 1,000 r.p.m. for 10 minutes and heated at 100° C. for 20 minutes or treated with phenol in 0.5 percent concentration. The second antigen consisted of heated and phenolized yolk sac material from infected chick embryos.

Complement Fixation Test

The procedure for the complement fixation test was as follows:

Serial fourfold dilutions of heated inactivated serums were made in veronal buffered saline. The first dilution varied from 1:2 to 1:8, depending upon the quantity of serum available. The serum dilutions were distributed in 0.1-ml. volumes to 3 rows of tubes. The first row was the test row and received 0.1 ml. of psittacosis antigen in optimal dilution. The second row served as a control for nonspecific reactions and received 0.1 ml. of normal allantoic fluid in the same dilution. The third row measured anticomplementary activity of the serum; it was given 0.1 ml. of veronal buffered saline.

To each tube was added 0.2 ml. of complement diluted to contain 1.5 to 2 units. The tubes were placed in the refrigerator overnight. Following this, 0.2 ml. of sensitized sheep cells (consisting of equal volumes of 2 percent cells and optimally diluted hemolysin) were added and the tubes incubated at 37° C. for 30 minutes. The titers represent serum dilutions which gave 3+ or 4+ readings followed by readings of "zero." When a 4+ was followed by a 1+ or 2+ reading, the titer was interpolated as being midpoint between the two dilutions. For example, if 1:16 dilution gave a reading of 4+, and 1:64 read 2+, the titer was considered to be at 1:32.

Each test was accompanied by the usual positive, negative, hemolytic system and cell controls. Unpublished results obtained in this laboratory indicate that human serums can be tested successfully with either psittacosis or lymphogranuloma venereum (LGV) antigens, but parakeet serums tended to react more specifically with psittacosis antigen in the complement fixation test. The psittacosis antigen may be preferable, therefore, for the testing of bird serums.

The indirect complement fixation test was performed by incubating at 37° C. for 1 hour 0.1 ml. each of the test serum, complement (2 units), and antigen (2 units). After this 0.1 ml. of a psittacosis immune mammalian serum (2 units) was added and the mixture again incubated for 1 hour at 37° C. Finally 0.1 ml. of 4 percent sensitized sheep cells was added and readings were made after 45 minutes incubation at 37° C.

Each test was accompanied by a direct fixa-

tion control in which a negative mammalian serum was substituted for the mammalian psittacosis positive serum. The usual controls for nonspecific, anticomplementary, and hemolytic reactions were also included for each test serum. Any serum which inhibited the fixation of complement by the known positive serum as indicated by readings of "zero," 1+, or 2+ was considered positive even if this reaction was observed only in the most concentrated dilution.

Virus Isolation

For virus isolations from excreta, the droppings from individually caged birds were suspended in a solution of 10-percent horse serum in buffered water, the proportions being approximately 1:3 by volume. After centrifugation at 300 times gravity for 10 minutes, the supernate was removed. Streptomycin sulfate was added in concentrations of 2.0 mg. per ml. of supernate, and the mixture was inoculated in volumes of 0.3 ml. intraperitoneally into groups of 3 to 5 mice which were 3 to 4 weeks old. Psittacosis virus was considered isolated when the mice developed ascites or died, with typical elementary bodies being demonstrated in smears from the peritoneal serosa.

When testing for virus in the tissues, an approximate 20 percent suspension of the liver and spleen of each bird was prepared and inoculated intracerebrally in volumes of 0.03 ml. into groups of 5 mice. Demonstration of elementary bodies in the smears from the meninges following a typical course of symptoms and death was the criterion of virus isolation.

Results

The results observed from testing 130 parakeets for both the presence of specific direct complement-fixing antibody in their serum and for virus in their tissues are shown in table 1. There was agreement between the two tests in 85 of 125 birds (5 were unsatisfactory in the complement fixation test). Of 30 birds from which virus was isolated, the serum of 5 failed to give a reaction in the complement fixation test. It is possible that serums from these birds were taken early in the infection prior to the development of demonstrable antibodies. The serum of a sixth bird was anticomplementary. No virus was isolated from 35 of the 59 birds whose serums gave a positive complement fixation reaction. This could mean that the virus isolation test was not sensitive enough to detect the amounts of virus present at the time, or that these birds had actually recovered from psittacosis infection and were free of virus but still possessed circulating antibody.

Table 1. Psittacosis virus isolations from serologically positive and negative parakeets

Results	Serums reacting to—	
	Direct CF test	Indirect CF test
Negative serologically; no isolation	61	26
Positive serologically; isolation	24	23
Positive serologically; no isolation	35	35
Negative serologically; isolation	5	3
Total	125	87

Birds from 13 flocks failed to yield virus when their livers and spleens were tested (table 2). Fifty-five of the fifty-eight birds so represented also failed to show complement-fixing antibody in their serums. Both tests yielded

Table 2. Comparison of virus isolations made from flocks with and without antibodies to psittacosis

Flock No.	Number parakeets tested	Number CF positive	Number virus isolations
1	24	21	15
2	5	2	2
3	3	1	0
4	8	1	0
5	5	2	1
6	4	4	3
7	4	1	0
8	5	2	4
9	25	25	4
10	5	0	0
11	11	0	0
12	3	0	0
13	3	0	0
14	4	0	0
15	3	0	0
16	4	0	0
17	1	0	0
18	3	0	0
19	6	0	0

positive results in 6 aviaries. The degree of correlation varied from 15 virus isolations versus 21 positive complement fixation tests (aviary 1) to 4 isolations versus 25 positive complement fixation tests (aviary 9). These findings may signify differences in the duration of infection in these 2 aviaries.

From flocks known to be infected, 74 parakeets were selected for a comparison of results of virus isolation from the droppings taken during life and from the liver and spleen at autopsy when sacrificed. Several samples of droppings were tested from some of these birds; only single samples were tested from others. Serum specimens were taken from all of the birds for parallel serologic studies. The results are shown in table 3.

Of the 23 parakeets in which tissues yielded psittacosis virus, only 13 shed virus in their droppings. The age or sex of the bird did not appear related to the excretion of virus. Virus was isolated from the droppings of one young female parakeet although no virus was obtained from its liver and spleen upon autopsy. In most cases repeated samples of droppings were taken at weekly intervals. Only 2 of the 10 birds which gave evidence of virus in any of three fecal specimens were excreting detectable amounts in all three samples.

The five birds with serums that gave positive

Table 3. Correlation of the presence of psittacosis virus in tissues and droppings and complement fixation antibody titers in individual parakeets

Parakeet No.	Age	Sex	Virus in tissue	Virus in droppings			CF antibody titer	Parakeet No.	Age	Sex	Virus in tissue	Virus in droppings			CF antibody titer
				1	2	3						1	2	3	
1	y	f	—	—	—	—	<1:8	38	y	nd	—	—	—	—	<1:2
2	y	f	—	—	—	—	<1:8	39	y	nd	+	+	—	—	<1:8
3	y	f	—	—	—	—	<1:8	40	A	f	+	—	—	—	<1:2
4	y	f	—	—	—	—	<1:8	41	A	f	—	—	—	—	1:32–1:64
5	y	m	—	—	—	—	<1:8	42	y	f	—	—	----	----	<1:8
6	y	nd	—	—	—	—	1:32	43	A	f	—	—	----	----	<1:4
7	y	nd	+	—	—	+	1:32	44	A	f	—	—	----	----	<1:8
8	A	nd	+	—	—	—	1:8	45	A	f	—	—	----	----	<1:4
9	y	nd	+	—	—	—	1:8–1:16	46	A	m	—	—	----	----	<1:4
10	y	nd	—	—	—	—	<1:8	47	y	m	—	—	----	----	<1:4
11	y	nd	+	—	—	—	1:8	48	A	f	+	+	----	----	<1:4
12	y	nd	—	—	—	—	1:8–1:16	49	A	f	+	—	----	----	<1:4
13	y	nd	+	—	+	+	[1] 1:128	50	A	f	+	—	----	----	1:16
14	A	m	+	—	+	—	[1] 1:128	51	y	f	—	—	----	----	1:2
15	y	m	+	+	+	+	[1] 1:128	52	y	m	+	—	----	----	<1:4
16	nd	nd	+	—	+	+	1:32	53	A	f	+	—	—	----	1:20
17	A	nd	+	—	—	+	1:8	54	A	f	—	—	—	----	[1] 1:40
18	y	f	+	+	----	----	AC	55	A	f	—	—	—	----	1:16
19	A	m	+	—	+	+	[1] 1:128	56	A	f	+	—	—	----	[1] 1:32
20	y	f	+	+	+	+	1:8	57	y	m	—	—	—	----	1:40
21	y	f	—	+	—	—	1:8	58	y	m	—	—	—	----	1:8
22	nd	nd	+	—	—	—	1:32–1:64	59	y	m	—	—	—	----	[1] 1:40
23	A	m	—	—	----	----	<1:8	60	y	m	—	—	—	----	1:4
24	A	f	—	—	----	----	1:8	61	y	m	—	—	—	----	1:8
25	A	f	—	—	----	----	1:8	62	A	f	—	—	—	----	1:8
26	A	m	+	+	----	----	[1] 1:128	63	A	f	—	—	----	----	[1] 1:64
27	A	f	—	—	----	----	1:32	64	A	m	—	—	----	----	1:5
28	nd	nd	—	—	—	—	1:4	65	A	m	—	—	----	----	1:5±
29	nd	nd	—	—	—	—	1:8	66	A	m	—	—	----	----	1:64
30	y	f	+	+	+	----	<1:8	67	A	f	—	—	----	----	1:16
31	nd	nd	—	—	—	—	<1:8	68	A	f	—	—	----	----	1:10
32	A	m	—	—	—	—	<1:2	69	A	m	—	—	----	----	1:16
33	y	nd	—	—	—	—	<1:2	70	A	m	—	—	----	----	1:40
34	y	f	—	—	----	----	<1:2	71	A	f	—	—	----	----	[1] 1:40
35	y	nd	—	—	—	—	<1:2	72	A	f	—	—	----	----	1:5
36	y	nd	—	—	—	—	<1:8	73	A	f	—	—	----	----	[1] 1:64
37	y	nd	—	—	—	—	<1:2	74	A	m	—	—	----	----	1:5

y=young; A=adult; nd=not determined; —=virus not isolated; +=virus isolated; f=female; m=male; AC=anticomplementary.

[1] Or greater.

complement fixation reactions at dilutions of 1:128 or greater were all shedding virus in their droppings. However, several which shed virus had CF titers of only 1:8, and three birds yielding virus from both droppings and tissues had no demonstrable CF antibodies. Possibly, the latter two groups of birds were in early stages of infection. Further studies on the pathogenesis and immunology of the disease in birds are necessary for proper interpretation and correlation of these data.

Of one flock of 49 birds examined by the complement fixation test, the serums of 25 reacted with psittacosis antigen. Ten of the reactors were removed and isolated from the flock 12 days later, and attempts were made to isolate virus from their droppings. Virus was recovered from only 2 of the 10. The remaining 15 reactors were then isolated from the flock 49 days after the original testing, and again virus was isolated from the droppings of 2 of these birds.

Fifty-five days after the first bleeding, second serum specimens were collected from the 24 birds which previously had shown no antibody. By this time all but one bird gave positive CF reactions with psittacosis antigen. Between the time of the first and second bleeding, breeding activities were initiated in this flock. This circumstance, plus the delayed removal of infected birds, may have been responsible for the apparent spread of infection within this flock.

A large group of young parakeets obtained from a wholesale dealer in New Jersey became available for study. Fifty of these birds were killed and their tissues tested for the presence of virus. Five of these yielded psittacosis virus. However, no antibody could be demonstrated in 197 serums by repeated direct complement fixation tests in which all controls indicated a satisfactory test.

These serums were then examined by the indirect complement fixation test. The type of correlation to be expected within an infected flock became apparent with the results obtained by this indirect test. Of 87 parakeets tested by both serologic and virus isolation techniques, 26 were negative by both tests, 23 were positive by both tests, 35 were serologically positive but failed to yield virus, while 3 were serologically negative yet virus was isolated from their tissues (table 1).

Summary and Conclusions

In the parakeets studied in this series, a close correlation was demonstrated between the results of complement fixation tests for psittacosis antibody and of virus isolation from tissues. Psittacosis virus was isolated from 47 of the 117 birds showing either direct or indirect complement fixation antibody titers of 1:2 or greater. Virus was not isolated from 87 of 95 parakeets whose serums contained no demonstrable antibodies. Most of the birds with antibody from which virus could not be isolated were obtained from known infected flocks. Conversely, only the serums of 3 of 55 birds obtained from flocks considered to be free of psittacosis virus (on the basis of a 10 percent sampling) reacted positively to the complement fixation test and then only in a low titer. A larger sample might have indicated that virus was present in the two flocks from which these birds came.

The significance of a positive titer in relation to the infectiousness of an individual bird remains to be determined, but the finding of at least some birds with antibodies may be regarded as evidence of past or present infection in that flock. Such a flock should be suspected and the birds with negative serums should be retested in 3 to 6 weeks. The presence of additional birds with antibodies at this time would provide presumptive evidence that active infections were present in the aviary. Continued absence of antibodies in a flock may be considered an indication of absence of current active infection.

The reason why some birds develop antibodies that can be detected in the direct complement fixation test while others develop only indirect complement-fixing antibodies is not understood. However, the results indicate that parakeet serums should be subjected to both tests before being considered negative.

Although isolation of virus will remain the most conclusive method for the diagnosis of psittacosis in individual birds, this expensive and time-consuming procedure need not be applied for the detection of infection in flocks.

Where facilities for virus isolation do not exist, or the number of birds to be tested is excessive, the complement fixation test should prove to be especially valuable. Aside from being one of the more reliable of the complement fixation tests for virus, it is relatively rapid and simple. This test should be added to the diagnostic armamentarium of every local public health laboratory.

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Increase in Juvenile Delinquency

The Children's Bureau, Department of Health, Education, and Welfare, has been receiving an increasing number of requests for help from States and communities who are trying to plan services for delinquent children more effectively.

The Bureau reported on May 20 that about a half million children were brought to juvenile courts for delinquency in 1955 and that juvenile delinquency continued to increase during that year. The Bureau, which annually receives reports from a number of juvenile courts on the juvenile delinquency cases they handle, has prepared preliminary estimates of the rates of increase.

Some 977 courts reporting to the Bureau in both 1954 and 1955 experienced a 9-percent increase in juvenile delinquency cases over that period. Likewise, a trend group of 383 courts which have been reporting for many years also show a 9-percent increase in the 1954–55 period. While the increases in cases were occurring, the child population in the 10–17 age group went up only about 3 percent. Generally, this is the age group within the jurisdiction of the juvenile court.

Not all children brought to attention of the police have committed offenses serious enough to warrant court action. The 9-percent increases in juvenile court cases may understate the actual increase in the number of juvenile delinquents in 1955. For example, the latest Federal Bureau of Investigation uniform crime report shows an 11.4 percent increase in police arrests of young persons under 18 in 1955 as compared with 1954. The FBI report is based on data from 1,162 cities.

Hopefully, joint efforts of local, State, and Federal groups can pull the delinquency rate down. Toward that end, consultants in the Bureau's Juvenile Delinquency Service Division are working with local communities and States in planning prevention and treatment programs.