Direct Complement-fixation Test for Diagnosis of Ornithosis in Turkeys.* (22608)

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The presence of ornithosis antibodies in turkeys has been known since 1942(1). The importance of turkeys as a source of human infection was reemphasized in 1948, and thereafter, by outbreaks of psittacosis among employees of turkey dressing plants(2,3) and by the subsequent isolation of a highly toxigenic ornithosis turkey strain(4). More recent studies have revealed the widespread occurrence of the virus in turkeys, and have led to the recognition of epizootics of varying degrees of severity which have been responsible for human infections. In view of the foregoing, rapid and sensitive methods for the detection of this disease in fowls are required.

The complement-fixation (CF) test has not been applied in the study and diagnosis of turkey ornithosis since, among other avian sera, Rice(5) reported the failure of antipullorum turkey sera to fix complement with *S. pullorum*, and Eddie and Francis(1) observed fixation at only low dilutions with a limited number of turkey antiornithosis sera. The presence of a specific inhibitor, presumably an incomplete type antibody, was demonstrated in avian non-complement fixing sera (5,6), and thus the indirect complement-fixation test (ICF) was devised for the detection of such antibodies(5,6). Hilleman and Helmold(7) suggested the use of the ICF for fowl antiornithosis sera, and Karrer, *et al.*(8) studied the variables involved in this test and applied the method for epidemiological surveys of ornithosis in chickens, ducks and turkeys(9). Since that time, the ICF has been used almost exclusively for the routine serological testing of turkey sera.

A previous communication from this laboratory described the extraction and characterization of soluble CF and skin-test antigens from the psittacosis virus(10). The CF antigen was found to fix complement consistently in the presence of turkey antiornithosis sera. Considering the ease of performing the direct complement-fixation test (DCF) when compared to the indirect method, an evaluation of the DCF was undertaken.

Materials and methods. The method of preparation of the detergent-extracted antigen was described previously(10). Meningopneumonitis virus (Francis) was used exclusively in the present study. To maintain maximum CF activity, the crude extract was cleared by relatively slow speed centrifugation (10,000 x G for 20 minutes), and used without further purification. The detergent extract could not be used for the ICF with sera giving positive DCFs, since direct fixation interfered with the inhibition reaction. Therefore for the ICF, these sera were tested with either a concentrated heated phenolized meningopneumonitis allantoic fluid suspension or a psittacosis (stain 6bc) yolk-sac particle suspension.

Sera. Groups of turkey sera were obtained from the following sources: A) year-old Broadbreasted Bronze turkeys from Oregon which had experienced a severe ornithosis epizootic approximately 3 months prior to bleeding;† B) 2- to 5-year-old Broadbreasted Bronze turkeys from the same ranch as flock A, but in which ornithosis was not evident;† C) a flock from the same vicinity as A, but without evidence of ornithosis infection;† D) sera from birds that had undergone a natural infection 18 months prior to bleeding;‡ E)

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‡ Kindly supplied by Dr. Raymond Bankowski, School of Veterinary Medicine, University of California, Davis.

TABLE I. Comparison of Direct and Indirect Complement-Fixation Tests for Detection of Turkey Ornithosis.

Group	History of infection	No. turkeys tested	DCF* pos ICF† "	DCF pos ICF neg	DCF neg ICF pos	DCF neg ICF "	% positive DCF	% positive ICF
A	Natural (3 mo)	47	41	3	0	3	93.6	87.3
B	No evidence	61	1	1	0	59	3.2	1.6
C	" "	50	2	0	0	48	4.0	4.0
D	Natural (18 mo before)	12	7	5	0	0	100	41.6
E	Exp. infected 8 wk	11	7	0	2	2	63.7	81.8
	Total	181	58	9	2	112	36.0	33.1

* Direct complement-fixation. † Indirect complement-fixation.

one-year-old Beltsville white turkeys experimentally infected with ornithosis virus (strain J.O.) by the intratracheal route 8 weeks before testing.§

Serological methods. The ICF was performed similarly to the method described by Karrer, *et al.*(8). For the DCF, 4 units of the detergent-extracted antigen are used. The preliminary incubation in both tests was carried out at 37°C for 75 minutes, and after the addition of sensitized sheep erythrocytes, a secondary incubation at 37°C for 30 minutes was made. Titers represent the reciprocal of the highest dilution of serum showing either a 1+ or 2+ reaction in the ICF (partial inhibition) or a 4+ reaction (complete fixation) in the DCF.

Results. When it was determined that the crude detergent extract would fix complement with randomly selected turkey antiornithosis sera, a survey of naturally infected and experimentally infected turkeys was made using the DCF. In some cases the ICF and DCF were compared. Forty-seven turkeys were selected at random from a flock that had undergone a severe epizootic several months prior to bleeding. Table I shows that 41 sera in this group (A) were positive by both the ICF and DCF, and that 3 sera were positive in low dilutions (1:4, 1:4, 1:16) in the DCF and negative in the ICF. Dr. B. Eddie of The George Williams Hooper Foundation, University of California Medical Center, San Francisco, kindly tested these sera by the DCF, and they were all negative to 4 units of a heat-killed psittacosis (strain 6bc) yolk-sac antigen. The significance of this observation will be discussed later. Also shown in Table I are results with sera from 2 flocks (B,C) in which there was no evidence of infection at the time of bleeding. The large number of negative sera attest to the specificity of the DCF. One serum from flock B was negative by the ICF and had a DCF titer of 1:4. To determine the duration of the DCF antibody, sera were obtained from 12 turkeys which had undergone a natural infection 18 months prior to the test. Only 5 sera had positive ICF titers; whereas, all sera had antibodies as revealed by the DCF (Table I).

Table II presents DCF titers of 20 turkeys experimentally infected for 8 weeks, and a comparison of the DCF and ICF titers of 11 of these sera. Two of the negative DCF sera were positive in the ICF. These findings sug-

TABLE II. Complement-Fixation Reactions of Turkeys Experimentally Infected 8 Weeks with Ornithosis.

Turkey	Titers: Direct test	Titers: Indirect test
9	8	32
18	4	8
46	16	8
63	32	32
64	256	16
85	0*	0
89	0	4
105	32	8
106	32	16
117	0	0
136	0	2
10	128	—
26	2	—
48	32	—
77	64	—
101	256	—
116	128	—
123	64	—
125	32	—
141	32	—

* Negative at 1:2 dilution.
— = Not tested.

§ Kindly supplied by Dr. Don Davis, A. and M. College of Texas, College Station.

TABLE III. Appearance of Complement-Fixing Antibodies in Experimentally Infected Turkeys.

Turkey	Days after infection 0	8	14
1	0*	2 (3+)	64
2	0	16	32
3	0	2 (3+)	8
4	0	2	64
5	0	4	8

* Negative at 1:2 dilution.

gested that if incomplete CF antibodies were formed in turkey sera, they might have been predominant during the early stages of the infection. However, on the contrary, positive DCFs were demonstrated 1-2 weeks following infection. Five one-year-old Beltsville white hens were infected with over 10^7 LD_{50s} (yolk-sac titration) of psittacosis virus (Texas turkey strain). All turkeys were negative prior to infection. Eight days following infection all turkeys showed DCF titers, and the titers increased by 14 days of infection (Table III). Thus, the earliest appearance of the DCF antibodies paralleled the reported appearance of the ICF antibodies in chickens (9,11).

To determine the relationship between the titers obtained by the 2 methods, a direct correlation analysis on the basis of the log distribution of the ICF and DCF titers of flock A was made.‖ Fig. 1 shows the regression line obtained, and the correlation was found to be 0.72, which was significant at the 1% level. Although there is no direct evidence as yet that the same antibody was being measured by the 2 tests, the DCF titers correlated well with the ICF titers of these sera. Prozones rarely occurred and then only in the 1:2 dilutions. Of the 91 positive sera examined to date, only one manifested an extended prozone. Attempts to eliminate the prozone of this one serum by extending the fixation period at 5°C failed. Employing the detergent-extracted antigen, attempts to obtain direct fixation of complement with chicken antisera met with repeated failures. Under the test conditions, chicken antibody truly represented the type that fails to fix complement, although chicken antiornithosis sera will precipitate with suitable antigens(12).

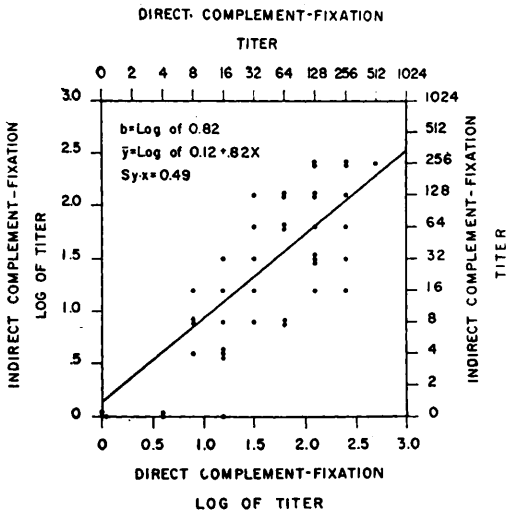

FIG. 1. Correlation analysis of direct and indirect complement-fixation reactions with turkey antiornithosis antibody.

In the light of previous claims that turkey sera fixed complement poorly(5,8), the consistency and sensitivity of the DCF in this study was surprising. The results suggested that successful fixation depended, in part, on the type of antigen used in the test. To test this hypothesis, a comparison was made of the detergent extract and other antigens commonly used in the DCF. A heated phenolized psittacosis (strain 6bc) yolk-sac antigen, a viable concentrated meningopneumonitis virus suspension (obtained by sedimentation of the virus from allantoic fluid), and the detergent extract were each titrated against 4 units each of rabbit and turkey antipsittacosis sera. The results summarized in Table IV reveal that the soluble antigen had the same titer when tested against rabbit and turkey sera. On the other hand, the allantoic fluid and yolk-sac antigens were considerably less active when titrated with turkey than with rabbit antiserum. The possibility existed that the detergent extract consisted of an antigen that differed either quantitatively or qualitatively from that found in the particle suspensions. Centrifugation of the soluble antigen at 35,000 x G for 30 minutes was known to sediment a CF antigenic fraction(10); thus, the

‖ The authors gratefully acknowledge the efforts of Miss Peggy Stimmel of this Department for the statistical analysis.

TABLE IV. Complement-Fixing Activity of Detergent Extract and Viral Suspensions when Tested with Rabbit and Turkey Antipsittacosis Sera.

Antigen	Psittacosis antiserum Rabbit*	Turkey*
Crude detergent extract		
AP	32†	32
AQ	64	64
Crude detergent extract ultracentrifuged		
Supernate	16	16
Pellet	8	8
Living meningopneumonitis allantoic fluid suspension	128	16
Killed psittacosis yolk-sac suspension	256	8

* 4 units of antiserum. † CF titer of antigens.

pellet and supernatant fractions obtained by this treatment were each titrated against rabbit and turkey antisera. Table IV shows that activity of the pellet antigen equals activity of the supernate antigen with rabbit or turkey antiserum.

Discussion. Employing the yolk-sac cocto antigen (6bc), Karrer *et al.*(9) demonstrated inhibition titers with a small number of pigeon sera which gave negative results in the DCF. These investigators stated that the inhibiting and DCF antibodies rarely occur in the same serum, and that this was evidence that the inhibiting and complement-fixing antibodies were different. Our studies show, however, that when using the detergent-extracted antigen, the DCF was as accurate as the ICF for the detection of ornithosis antibody in the turkey sera studied, and that with some sera the DCF was a more sensitive indicator than the ICF. Furthermore, all but a few positive sera gave both positive indirect and direct CF reactions. These conflicting observations can be explained on the basis of the types of antigens employed. Direct fixation with turkey sera would be missed if the virus suspension antigens were standardized with non-avian or pigeon sera and then the usual 4 units of antigen were used in the direct test with turkey sera. When tested with rabbit antiserum, 4 units of the allantoic fluid and yolk-sac antigens shown in Table IV represent titers of 32 and 64, respectively; concentrations that were insufficient to fix complement with turkey sera. At least for turkey antisera, these data suggest that the inhibiting and complement-fixing antibodies may represent the same antibody, and that their detection partially depends upon the molecular configuration of the antigen. The ability of the detergent extract to fix complement with turkey antibody may be due to the physical state of the antigen. The reactive sites of the soluble antigen may be more readily available, and the union with antibody and complement is probably firmer than that found with the virus preparations. The failure to detect serious prozone effects, the early appearance in infection of the DCF antibodies, and the detection of antibodies in turkeys infected 18 months previously strengthen the practical use of the DCF.

Summary. Employing a soluble antigen, complement-fixation reactions were obtained with turkey antiornithosis sera. Antigens consisting of viable allantoic and killed yolk-sac virus suspensions did not show the same degree of activity. The direct complement-fixation reaction was as sensitive as the indirect complement-fixation test; therefore, the use of the direct test for the study and diagnosis of turkey ornithosis was suggested.

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