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Occurrence of Psittacosis-like Infection in Domestic and Game Birds of Michigan.*

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The latent presence of a form of psittacosis in pigeons, called by Meyer, ornithosis, has been demonstrated by Pinkerton and Swank,[1] Coles,[2] and Meyer, Eddie and Yanamura.[3] The characteristics of the virus indicate that it is related to the virus of typical psittacosis but resembles more closely the type of psittacosis virus found in Australian parrakeets.[4] Moreover, available evidence points to the similarity of its pathogenic characteristics and those of the virus of meningo-pneumonitis (M-P) originally described by Francis and Magill.[5] Recent serological studies have clearly shown that a close relationship exists between the viruses of psittacosis, meningo-pneumonitis and lymphogranuloma venereum[6, 7] and that patients infected with one of the viruses produce antibodies which fix complement in the presence of any one of the 3 antigens.

That the virus of pigeon origin may infect human subjects is shown by actual recovery of the virus from patients,[6] some of whom were intimately associated with infected birds.[3] These results have heightened the interest in the pigeon as a disseminator of disease and the recent report of Meyer, Eddie, and Yanamura[3] has furnished evidence of the wide distribution of infection in this species of bird. Studying pigeons from California, New York, Indiana, Iowa, and South Carolina, they found that the sera from approximately 50% of the 237 pigeons tested fixed complement to some extent in the presence of psittacosis antigen and from 25 of the pigeons virus of ornithosis was actually recovered.

* This study was conducted under a grant from the International Health Division of the Rockefeller Foundation.

1 Pinkerton, H., and Swank, R. L., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 704.

2 Coles, J. D. W. A., *Onderstepoort J. Vet. Sci. and An. Ind.*, 1940, **15**, 141.

3 Meyer, K. F., Eddie, B., and Yanamura, H. Y., Proc. Soc. Exp. Biol. and Med., 1942, **49**, 609.

4 Meyer, K. F., and Eddie, B., *J. Inf. Dis.*, 1939, **65**, 234.

5 Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1938, **68**, 147.

6 Eaton, M. D., Beck, M. D., and Pearson, H. E., *J. Exp. Med.*, 1941, **73**, 641.

7 Rake, G., Eaton, M. D., and Shaffer, M. F., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 528.

In view of this evidence the present study was undertaken to ascertain the distribution of ornithosis in the avian species of Michigan.

1. *Complement-fixation Test.* (a) *Preparation of Antigen.* In this study the antigen was prepared in 100 cc amounts, a total of 1100 mouse lungs being used. Fifty mice were inoculated intranasally with 0.05 cc of a 10% lung-suspension of the highly virulent F97 strain of meningo-pneumonitis virus. After 45 to 48 hours the lungs were removed from the mice aseptically and ground in a mortar with physiological saline (pH 7.6), approximating 2 cc of saline per lung. The procedure followed thereafter was the same as that employed in the preparation of psittacosis antigen.[8] Exactly the same procedure was followed in the preparation of antigens from normal mouse lungs which were used for controls.

(b) *Serum* was obtained from blood taken aseptically from the ulnar vein of the wing or if the bird was to be sacrificed, blood was taken from the heart.

(c) The test was performed in essentially the same manner employed for the diagnosis of psittacosis.[8] Two *full* units of complement were used in the tests here reported. The hemolytic system consisted of washed sheep cells suspended in a dilution of amboceptor so that 0.5 cc contained 2 units of amboceptor and a 2% suspension of red blood cells. The end point adopted was the highest dilution in which complement was completely fixed.

As a rule, each serum was titrated against both infected and normal lung-antigen. Owing to the fact that human sera give cross reactions with the antigen of lymphogranuloma venereum, a certain number of tests were made with the latter antigen as well.

2. *Isolation of Virus.* In each instance, groups of 3 or 4 mice were inoculated intraäbdominally with 10% suspensions of liver and spleen, kidneys and ovaries or testes, and 1% intestinal contents, respectively, from a single pigeon. The mice were kept in isolated quarters. After an interval of 21 to 30 days all surviving mice were sacrificed and passage was made with suspensions of liver and spleen intranasally to normal mice. After a similar interval an additional passage with suspensions of the lungs of these mice was made intranasally. If no abnormalities were detected after 3 transfers, the series was discontinued. If pulmonary lesions were found the passages were continued until virulence was enhanced. Impression-smears from the lungs were made, stained by the Machiavello

[8] Meyer, K. F., and Eddie, B., *J. Inf. Dis.*, 1941, **68**, 1.

technic[9] and examined for elementary bodies. The characteristic pulmonary pathology also served in identification of the agent.

The tests were controlled by the simultaneous maintenance of mice inoculated with non-infectious material under the same conditions.

Results. (a) *Pigeons.* The pigeons were obtained as follows: 24 from the Detroit Market; 24 from loft J. S., 12 from loft J. C., Ann Arbor; 20 from loft K in East Lansing; 18 from loft Dr in Lansing. The results of the complement-fixation tests are summarized in Table I. It is seen that positive reactions were obtained with 61% of the sera. The general incidence corresponds with that reported by Meyer, Eddie, and Yanamura[3] in other states. The percentage of positive reactions among the specimens from different groups ranged from 8.5 to 85. No significant difference in the number of reactors could be attributed to sex, age, or the size of the pigeon's spleen.

It is of interest to record that while a marked parallel is obtained in the reaction of the serum of human subjects to the viruses of meningo-pneumonitis, psittacosis, and lymphogranuloma venereum, pigeon serum exhibits a greater specificity. The reaction to antigen of typical psittacosis was essentially the same as to M-P antigen.

TABLE I.
Complement-Fixation Test with Serum of Pigeons in Michigan.

Titer	Detroit Market 1-13-42	Ann Arbor Loft J.S. 12-24-41	Ann Arbor Loft J.C. 1-9-42	Ann Arbor Loft J.S. 4-2-42	E. Lansing Loft K. 3-26-42	Lansing Loft Dr. 3-26-42	Total
0	9	3	8	5	3	5	33
1:2	1	1	1	0	1	1	5
1:4	0	1	0	0	1	1	3
1:8	0	0	0	1	2	2	5
1:16	1	1	0	0	3	2	7
1:32	2	2	0	1	5	3	13
1:64	1	2	0	3	2	2	10
1:128	4	2	0	4	2	2	14
1:256	3	0	0	0	1	0	4
Anticomplementary	3	0	3	0	0	0	6
Total	24	12	12	14	20	18	100
No. of reactors	12	9	1	9	17	13	61
% reactors	50	75	8.3	64	85	72	61
No. tested		12	12	14			36
Virus recovered	*	1	1	Incomplete	*	*	2

*Not attempted.

9 Machiavello, A., *Virus and Rickettsial Diseases,* Harvard Univ. Press, Cambridge, Mass., 1941, p. 896.

But the sera of pigeons with high titers of complement-fixing antibodies to M-P virus failed to react with antigen prepared with the virus of lymphogranuloma venereum. These observations indicate that factors other than antigenic constitution play a rôle in determining the cross-reactions obtained in serological tests. They suggest, furthermore, that the use of pigeon serum may be of value in the differentiation of this group of infectious agents.

In addition, attempts were made to isolate virus from 36 individual pigeons irrespective of the serological status. Virus was recovered from the liver and spleen of one pigeon, and from the ovaries and kidneys of another (Table I). The virus appears in all respects to be typical of the virus of ornithosis in pigeons (Meyer). The fact that material from 34 additional birds studied simultaneously yielded no virus appears to eliminate the possibility that the virus was resident in the stock of mice as reported by Nigg.[10] Control passages have likewise been negative.

(b) *Other Domestic and Wild Fowl.* Sera were obtained from 109 domestic turkeys and 45 chickens in different parts of the state and from 24 domestic ducks at the Detroit market. Sera from 55 wild ducks, 25 ringneck pheasants and 10 Hungarian partridges were also obtained (Table II). With 22 of the turkey sera fixation was observed in dilutions ranging from 1:4 to 1:16. Of the chicken

TABLE II.
Complement-Fixation Test with Serum from Domestic and Wild Fowl in Michigan.

	Domestic			Wild		
Titer	Chickens	Turkeys	Ducks	Hungarian Partridges	Pheasants	Ducks
0	36	67	15	10	25	55
1:2	2+ 4+ 3+++	1++++ 3+	1++++	0	0	0
1:4	0	8++++ 2+++ 2++ 4+	1++++	0	0	0
1:8	0	1+++ 2++	0	0	0	0
1:16	0	2++++ 1++	3++++	0	0	0
Anticomplementary	0	16	4	0	0	0
Total	45	109	24	10	25	55

[10] Nigg, C., *Science*, 1942, **95**, 49.

sera tested, 9 gave a reaction in the lowest dilution. With 5 of the sera of domestic ducks, reactions in serum dilutions of 1:2 to 1:16 were obtained. Tests with the sera of the wild fowl were completely negative. No attempts to recover virus were made. The results suggest that infection with ornithosis occurs in domestic turkeys and ducks while game birds, at least in Michigan, are relatively free of the infection.

Summary. Complement-fixation tests with the virus of meningopneumonitis in Michigan revealed that 61 of 100 pigeons reacted with the antigen; 41 of them in dilutions of 1:32 or greater. Pigeon serum fails to give the cross-reaction with lymphogranuloma venereum antigen which is commonly seen with human serum.

Reactions of variable intensity were obtained with 22 of 109 sera from domestic turkeys, with 5 of 24 sera from domestic ducks and, in the lowest dilution only, with 9 of 45 chicken sera.

No reactions were observed with the sera of 90 wild fowl.

The authors wish to express their appreciation for aid in obtaining blood samples from the various sources to Dr. H. J. Stafseth, Michigan State College; Dr. Stanley Whitlock, Michigan Department of Conservation; Mr. W. F. Van Dien, Director of Michigan State Game Farm; and Dr. J. G. Molner, Deputy Commissioner of Health, Detroit, Michigan. The psittacosis antigen was kindly furnished by Dr. K. F. Meyer of the Hooper Foundation, San Francisco; the lymphogranuloma antigen by Dr. Clara Nigg of the Squibb Institute, New Brunswick, New Jersey, to whom we express our thanks.

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Sinus Gland Extirpation in the Crayfish Without Eyestalk Removal.

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In view of the increasing volume of literature pertaining to endocrine activities of the crustacean sinus gland, it is unfortunate that the only simple and sure method of removal of these glands from the body has involved the removal of the total eyestalk. The eyestalk constituting the most important light receptor of the organism, contains, in addition to the sinus gland, several important nerve ganglia, the x-organ, and other possible endocrine sources. For this reason the usual removal of so much additional tissue with the glands leaves