Comparison of the Indirect and "Implemented" Direct Complement–Fixation Test in the Diagnosis of Turkey Ornithosis

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The increased number of reports on the public health importance of turkey ornithosis has stimulated investigations of serological methods for the diagnosis of this infection. In 1947, Rice (1) showed that heat inactivated turkey anti-pullorum sera failed to fix guinea-pig complement with *S. pullorum* antigen. The testing of non inactivated sera being unsatisfactory because of their anticomplementary and non-specific properties, Rice developed an indirect complement-fixation method for the demonstration of avian serum antibodies (2). Similar types of tests were adapted to the detection of ornithosis avian antibodies by Karrer, Meyer and Eddie (3) and also by Hilleman, Haig and Helmold (4).

A variety of other serological tests have been developed for the detection of ornithosis avian antibodies. Brumfield and Pomeroy (5) have developed a direct complement-fixation test in which the guinea-pig complement is "implemented" with normal unheated chicken serum. The normal serum is added to supply an essential heat-labile factor which is destroyed during heat-inactivation of the test serum.

Our study was undertaken to compare the efficacy of the indirect complement-fixation test as used in our laboratory with the direct test incorporating the modification of Brumfield and Pomeroy (5) in the diagnosis of turkey ornithosis.

Materials and Methods

Turkey Test Sera

Three categories of test sera were compared: control, experimental and sera from field outbreaks.

Control sera. Eight sera were sent to us by Dr. H. Brumfield, Minnesota University and were used at first, as controls in our test. Five of these sera were collected from field cases of infection, two from hyperimmunized birds and one from a normal turkey.

Experimental sera. Control sera were produced experimentally in three groups of turkeys. Two turkeys (357-358) were inoculated intratracheally with 1.5 ml. of a 20 per cent broth emulsion of mouse spleen infected with a strain of ornithosis virus isolated from a budgerigar A.D.R.I. — P2. These birds were bled before inoculation and again in 3, 5, 6, 10 and 12 weeks. They were re-inoculated after the bleeding of the third week and one of them received a third inoculation at the 7th week.

Five turkeys (370, 371, 373, 374) were inoculated by the same route with 2 ml. of a similar emulsion. They were bled before and at the 3rd and 5th week after the first inoculation. They were re-inoculated after the bleeding of the 3rd week.

Another five turkeys (378, 379, 380, 381, 17694) received 1.5 ml. of similar viral suspension. They were bled before inoculation and 1, 2, 3, 5, 7 and 11 weeks later. The sera from the three lots of birds were frozen soon after collection.

Field sera. Twenty lots, including 945 turkey sera sent to us by Dr. H. C. Carlson, Veterinary Pathologist, Edmonton, Alberta are included in this group. Some of these lots were collected on farms known to have turkeys infected with ornithosis virus. These sera when received were not frozen and some were contaminated with bacteria.

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TABLE I

Serological tests on control turkey ornithosis sera received from Dr. H. Brumfield

Control Turkey Sera		Complement-fixation Titres	
		Indirect	"Implemented" Direct
Hyperimmune	25052	640	160*
"	6222	320	160*
Field	159874	80	80
"	159884	80	80
"	159899	160	160
"	159984	160	160
"	34917	40	40
Normal	12269	—	—

*End point titre not determined.

Antigens

The same group-reactive antigen was used for both tests. It was prepared as previously described (6) from yolk sacs of embryonated chicken eggs inoculated with a strain of the virus of enzootic abortion in ewes that had been well adapted to grow in chick embryo. Pools from heavily infected yolk sacs were homogenized in a Virtis homogenizer and phenolized to a 5 per cent concentration. After standing in the refrigerator for a week, the phenolized suspension was homogenized with four volumes of beef heart broth of pH 7.0. The coarse particles were removed by slow centrifugation or standing. The supernatant after heating in boiling water bath for 30 minutes, constituted the antigen.

Complement-Fixation Methods

Before titration the turkey test sera were inactivated by heat at 56°C. for 30 minutes. The evaluation in both methods was based on the 50 per cent haemolytic unit, that is the amount of complement necessary to haemolyse 50 per cent of the standard volume of sensitized sheep red blood cells. The density of the cell suspension was adjusted by means of a photoelectric colorimeter. The reading of the degree of haemolysis was made with the help of a color standard prepared from the day's reagents. All dilutions were made in veronal buffered saline containing Mg and Ca ions.

Indirect complement-fixation test. The technique employed was a modification of the method described for the *S. pullorum* test (2). On the first day of the test, two-fold dilutions of the inactivated turkey test sera were mixed in 0.1 ml. amounts with 0.05 ml. of the antigen and incubated at 6 to 9°C for 18 hours. The antigen was employed in the highest dilution of which 0.05 ml. had given complete fixation of four and a half 50 per cent haemolytic units of guinea-pig complement in the antigen titration. On the second day of the test, 0.1 ml. of a complement dilution containing four and a half 50 per cent haemolytic units was added. This was followed by the addition of 0.05 ml. of a dilution of inactivated guinea pig serum containing an excess of ornithosis antibody. After mixing, the test was held at 6 to 9°C for additional 18 hours. On the third day, 0.2 ml. of maximally-sensitized 2.5 per cent sheep red blood cells were added and the test incubated at 37°C in a water bath for 30 minutes. Serum, antigen and complement controls were included with each test.

Direct complement-fixation test "implemented" with normal chicken serum. This test was based on the observations of Brumfield and Pomeroy (5) adapted to our routine method as described for the modified direct complement-fixation test for cattle serum (7). In brief, it consisted of delivering into the appropriate test tubes 0.1 ml. of two-fold dilutions of heat-inactivated turkey test serum. Then to each tube was added 0.1 ml. of a dilution containing four and a half 50 per cent haemolytic units of guinea-pig complement diluted in buffered salt solution supplemented with 5 per cent pretested normal fresh chicken serum. After the addition of 0.1 ml. of a dilution containing 2 units of antigen the mixture was incubated at 37°C for a 90 minute fixation period. Maximally sensitized 2.5 per cent sheep red blood cells were added in 0.2 ml. amounts and the test incubated at 37°C for a 30 minute haemolysis period. Serum, antigen and complement controls were included with each test.

Results

Control Sera

Close agreement was shown between the results of the indirect and the "implemented" direct complement-fixation tests on the two hyperimmune and five sera

TABLE II

Serological tests on sera from experimentally infected ornithosis turkeys

Infection	Turkeys	Titres at Weekly Intervals																	
		1		2		3		5		6		7		10		11		12	
Time		I.*	D.**	I.	D.	I.	D.	I.	D.	I.	D.	I.	D.	I.	D.	I.	D.	I.	D
1st, 3rd and 7th week	357					40	10	640	40	320	40								
	358					80	20	80	20	80	10			10	—			40	—
1st and 3rd week	370					640	40	640	40										
	371					80	10	640	80										
	372					x	160	640	80										
	373					80	10	320	40										
	374					160	20	640	10										
1st week	378	x	x	320	x	1280	x	640	80			160	40			x	160		
	379	x	40	320	x	640	40	1280	80			80	20			x	20		
	380	x	10	x	40	1280	80												
	381	160	5	640	10	1280	40	1280	160			1280	160			x	160		
	17694	—	—	—	—	—	—	160	10			10	5			x	80		

*I: Indirect complement-fixation test: **D: "Implement" direct complement-fixation test: x: not tested

TABLE III

Results of complement-fixation tests on 945 turkey field sera submitted for ornithosis tests

Indirect Titres	"Implemented" Direct Titres							Total
	Neg.	5	10	20	40	80	160	
Negative	704	2	10	1				717
5	8	1						9
10	97	1	13	1	1			113
20	29	2	13	10				54
40	3	1	2	17	3			26
80	2	1	2	6	3			14
160					1	2		3
320				3	3	1	1	8
Total	843	8	40	38	11	3	1	945

from field cases of ornithosis sent to us by Dr. H. Brumfield. The titres obtained in tests done shortly after reception are shown in Table I. Test performed at a later date on these sera showed a gradual decrease in titres in the "implemented" direct test. A year after the sera were received, being thawed and frozen a few times during this period, they were almost negative in the "implemented" direct test, whereas the titres in the indirect test were practically unchanged.

Experimental Sera

The results of both types of complement-fixation test on a series of turkeys used in the experimental production of control sera are given in Table II. The first two turkeys, one which received two and the other three infective doses, showed an increase in serum antibody titres in both tests after the first two infective doses. Serum titres of the second turkey decreased after the third dose. The second group of five experimental turkeys which received two infective doses, had high complement-fixation titres in both tests on bleedings taken the third and fifth weeks after primary infection. Titres of the same magnitude and even higher were obtained in the last group of five turkeys which received only one infective dose. Reactions were still of diagnostic significance seven and eleven weeks after inoculation. In general the titres obtained with the indirect test were 8 to 16 fold higher than those of the "implemented" direct test.

Field Sera

More discrepancies were seen between both tests with field sera, likely because these had not been kept frozen after collection and during transit. Some of them were tested only three or four weeks after collection and had undergone some degree of putrefaction. In Table III, out of 94 sera with indirect complement-fixation titre of 1:20, 1:40 or 1:80, there were 34 which gave a negative result in the "implemented" direct complement-fixation test. Conversely, only one serum among the 53 with an "implemented" direct complement-fixation titre of 1:20 or higher was negative in the indirect test. Sera with titres of 1:10 or lower which are of questionable diagnostic significance showed the greatest difference in results in both tests. Good agreement was obtained with high titre sera in the range of 1:160 or higher.

Discussion

Relative good agreement was obtained between the result of the two complement-fixation techniques on experimentally produced turkey ornithosis sera but the indirect complement-fixation test proved the more sensitive especially with field sera which were not frozen immediately after collection. However, the indirect test is more difficult to standardise and requires a complement-fixing serum which is at times difficult to produce. In the present study, this complement-fixing serum was obtained from guinea pigs infected with small inoculum of ornithosis virus in avian tissue. In addition to a high titre of ornithosis antibody these sera must not react with avian tissue. Only weakly fixing sera were obtained when the guinea pigs were

inoculated with infected mouse or guinea-pig spleen. Sera from naturally infected pigeons can be used if available in satisfactory titre. Positive sera from cattle infected with the enzootic abortion in ewes virus did not give as clear-cut a test as sera of guinea-pig origin.

The "implemented" direct complement-fixation test is a somewhat simpler test to standardize than the indirect test. However, a constant source of fresh normal frozen chicken serum should be kept available to "implement" the test. Positive sera, for control purpose are difficult to maintain due to their gradual deterioration upon storage. For best results the sera to be tested should be collected aseptically and frozen if the test is not done soon after collection.

Summary

The indirect complement-fixation test and the "implemented" direct complement-fixation test were compared on series of sera from turkeys undergoing natural field infection as well as from those with experimentally produced infections. Both tests gave comparable results on experimental sera that were frozen immediately after collection and stored in this way until time of testing. With low titre field sera which were not frozen and which were in transit for many days before being tested, greater discrepancies were obtained. However, high titre sera compared favorably in both tests.

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Muscle Relaxants in Veterinary Anaesthesia

It is well realized that surgical manoeuvres and diagnostic procedures are more easily carried out if muscle tone is reduced or abolished and that often one of the main purposes of anaesthesia is to achieve relaxation of the skeletal muscles. In spite of this awareness the value of the most effective type of therapeutic agent for the production of muscle relaxation-neuromuscular blocking agents-is largely ignored in veterinary practice. This paper is a review of the physiology of normal neuromuscular transmission and the pharmacological mode of action of neuromuscular blocking agents such as curare. The effectiveness of agents of this type-curare, gallamine triethiodide, laudexium methyl sulphate, suxamethonium in various domestic animals is discussed.

Several advantages accrue from the use of muscle relaxants in general anaesthesia. First, deep depression anaesthesia is rendered unnecessary. Second, immediate control of the degree of relaxation is insured. Third, immediate control of respiration is possible. Fourth, the establishment and maintenance of light anaesthesia without fear of respiratory spasm, cough or hiccup is facilitated. Fifth, ideal conditions are afforded with safety for intrathoracic surgery, intro-abdominal surgery and endoscopies, and owing to the profound relaxation which can be induced atraumatic endotracheal incubation is possible in pigs and cats. Sixth, in horses smooth induction of inhalational anaesthesia is possible in lightly narcotized animals.

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