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Specific Antibodies for *Mycoplasma* in Pheasants on Game Farms in Iowa

STEVEN PALMER and DAVID J. ROSLIEN

**Abstract:** Serums from 838 ring-necked pheasants (*Phasianus colchicus*) reared on 28 Iowa game farms were tested for *Mycoplasma gallisepticum* specific antibodies. Two hundred serums reacted with tube agglutination antigen, but only eight were confirmed with the hemagglutination-inhibition test.

**INTRODUCTION**

The purpose of our study was to determine the status of *Mycoplasma gallisepticum* infection in ring-necked pheasants reared on game farms in Iowa. The occurrence of avian *Mycoplasma* (Pleuropneumonia-like organism, PPLO) infections in pheasants on Iowa game farms had not previously been studied. Chronic respiratory disease (CRD) and air-sacculitis in chickens and infectious sinusitis in turkeys, diseases caused by avian *Mycoplasma*, occur in Iowa poultry. A study of the status of avian *Mycoplasma* infections in Iowa game farm pheasants appeared conducive to a more complete knowledge of both game farm management and pheasant biology.

Avian *Mycoplasma* infects chickens, turkeys, peafowl, ducks, partridges, and pheasants (Yoder 1963). The pathology of *Mycoplasma* infections in poultry is described by Hofstad (1959) and reviewed by Yoder (1963) and Yoder and Hofstad (1964).

Attempts to artificially infect ring-necked pheasants with avian *Mycoplasma* have been made, with varying success, by Jerstad and Hamilton (1948); Jungherr et al. (1952); Van Roekel et al. (1953); and McDiarmid (1960). Keymer (1958, 1961), in England, and Osborn and Pomeroy (1958), in the United States, reported natural *Mycoplasma* infections in game farm pheasants.

Serums from 95 Iowa wild pheasants checked by Andrews (1963) were negative for antibodies specific for pathogenic avian *Mycoplasma*. Five of 67 wild pheasant serums tested by L. A. Page at the National Animal Disease Laboratory, Ames, Iowa, in 1963, reacted (1:8 serum dilution) with avian *Mycoplasma* rapid plate antigen.

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2. Recipient of a grant from National Science Foundation Undergraduate Research Participation Program, Iowa State University, Ames, Iowa.
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Methods and Materials

Serums from 838 ring-necked pheasants were collected in 1962, 1963, and 1964. These serums (stored in the serum bank of the Iowa Cooperative Wildlife Research Unit, Iowa State University, Ames) were obtained from pheasants reared on 28 game farms in 17 Iowa counties (Figure 1). Sampling was repeated on two flocks in 1963 and 1964. Samples were collected June-October (Table 1). The gross physical condition of each bird was noted at the time of blood collection.

Table 1. Monthly distribution of pheasant serums and HI reactors for Mycoplasma.

<table>
<thead>
<tr>
<th>Month of collection</th>
<th>Flocks sampled</th>
<th>Pheasants sampled</th>
<th>Reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number Per cent</td>
<td>Number Per cent</td>
<td>Number Per cent</td>
</tr>
<tr>
<td>June</td>
<td>4 13</td>
<td>18 2</td>
<td>2 25</td>
</tr>
<tr>
<td>July</td>
<td>2 7</td>
<td>127 15</td>
<td>2 25</td>
</tr>
<tr>
<td>August</td>
<td>13 43</td>
<td>390 47</td>
<td>3 37</td>
</tr>
<tr>
<td>September</td>
<td>6 20</td>
<td>171 20</td>
<td>0 0</td>
</tr>
<tr>
<td>October</td>
<td>5 17</td>
<td>132 16</td>
<td>1 13</td>
</tr>
<tr>
<td>Total</td>
<td>30° 100</td>
<td>838 100</td>
<td>8 100</td>
</tr>
</tbody>
</table>

* Flock number includes two farms visited twice each during the study.

Tube Agglutination Test. All serums were first screened for Mycoplana gallisepticum specific antibodies with a tube agglutination test described by Yoder (1963). One ml antigen, diluted 1:20 with Cox phosphate buffered saline (pH 7), was mixed with 0.08 ml serum in a 75x100 mm test tube to attain a 1:12.5 serum dilution. Tests were incubated either at 56°C (water bath) for 4 hours and 8 hours at room temperature, or for 13-16 hours at 37°C (water bath).Tests were read after incuba-
tion and again after 24 hours at room temperature. Positive reactions were indicated by white flocs settled on the bottom of the test tube and clearing of the supernatant fluid.

**Hemagglutination Inhibition Test.** Positive or questionable serums were retested with the hemagglutination-inhibition (HI) test devised by Van Herick and Eaton (1945) and described by Yoder (1963). HI tests were conducted in plastic depression trays (Linbro). *Mycoplasma gallisepticum* serotype A (S6), isolate 801, hemagglutinating antigen was used at 2 hemagglutination units. Four cups in the depression tray were used for each test. To cup 1 was added 0.5 ml saline, and to cups 2-4 was added 0.5 ml antigen. Serums were dropped in 0.04, 0.02, 0.01, and 0.005 ml quantities with a brucella pipette to form 1:12.5 (saline control), 1:25, 1:50, and 1:100 serum dilutions. Each cup then received 0.5 ml 0.25% pheasant erythrocyte suspension. Positive and negative control serums were tested with each series of serums. Tests were incubated for 2 hours at room temperature.

An even distribution of erythrocytes over the bottom of the depression cup indicated a negative test. Formation of a compact “button” of erythrocytes at 1:50 (Fig. 2) indicated a reactor. Yoder (1963) considered a positive reading at 1:40 indicative of a reactor.

Both tube agglutination and hemagglutinating antigens were provided by the Iowa State Veterinary Medical Research Institute, Ames, Iowa.

Fig. 2. Positive hemagglutination inhibition test for *Mycoplasma* using a ten-fold serum dilution with positive and negative controls (c).
RESULTS

Two hundred of 838 serums gave positive or questionable reactions to the tube agglutination test. However, only 8 of the 200 serums retested with the hemagglutination-inhibition test reacted. Five of the eight reacted at 1:50 or higher; three reacted at 1:25 (Table 2). Reactors at 1:50 or higher were present in Pottawattamie, Lucas, Boone, and Warren counties (Fig. 1). Physical condition of the reactors ranged from good to poor (Table 2).

Table 2. Serum titer, sex, age, physical condition and size of parent flock of all reactors for Mycoplasma

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Serum titer</th>
<th>Sex</th>
<th>Age months</th>
<th>Physical condition</th>
<th>Flock size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:25</td>
<td>F</td>
<td>12</td>
<td>good</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>1:25</td>
<td>M</td>
<td>24</td>
<td>good</td>
<td>4</td>
</tr>
<tr>
<td>3*</td>
<td>1:25</td>
<td>F</td>
<td>12</td>
<td>good</td>
<td>356</td>
</tr>
<tr>
<td>4*</td>
<td>1:100</td>
<td>F</td>
<td>15</td>
<td>good</td>
<td>356</td>
</tr>
<tr>
<td>5</td>
<td>1:50</td>
<td>F</td>
<td>4</td>
<td>fair</td>
<td>5500</td>
</tr>
<tr>
<td>6</td>
<td>1:50</td>
<td>M</td>
<td>11</td>
<td>fair</td>
<td>120</td>
</tr>
<tr>
<td>7†</td>
<td>1:50</td>
<td>F</td>
<td>12</td>
<td>poor</td>
<td>8</td>
</tr>
<tr>
<td>8†</td>
<td>1:100</td>
<td>F</td>
<td>12</td>
<td>poor</td>
<td>8</td>
</tr>
</tbody>
</table>

* From same flock.
† From same flock.

DISCUSSION

Antibodies specific for Mycoplasma gallisepticum at high titer occurred in 5 of 838 pheasant serums tested by HI procedures. Serums from three other pheasants reacted at a questionably low titer (1:25). The first of these was probably positive since another bird in the flock had a significant titer. The remaining two questionable reactors occurred in negative flocks, and it is possible that a nonspecific HI reaction was responsible.

The large number of positive tube agglutination tests from the sample was probably caused by nonspecific reactions. Tube agglutination procedures are commonly used for screening large numbers of serums; however, hemagglutination inhibition procedures are more specific for avian Mycoplasma. HI reactors provide more evidence of exposure to avian Mycoplasma than do tube agglutination tests when isolates of Mycoplasma from the reactors are not available.

In one flock, reactors (Nos. 7 and 8, Table 2) were in poor condition. These pheasants were kept in a small pen with about 50 pigeons. The pigeons were unthrifty and may have been carriers of chronic respiratory disease similar to that described by Winterfield (1953). Although four of the five positive reactors were in poor-fair condition, symptoms do not always accompany infection. Fahey and Crawley (1954) and Dunlop et al. (1961) noted a lack of clinical symptoms in some poultry reactors.
Reactors were about evenly distributed throughout the months sampled (Table 1) and occurred in flocks of various sizes (Table 2).
Pheasants on one farm were negative despite prolonged contact with CRD-positive chickens. Pheasants may sometimes be refractory to Mycoplasma infection as was indicated by Jerstad and Hamilton who found that two pheasants penned with turkeys with infectious sinusitis did not become infected, although other turkeys did (1948).
Serological tests may not detect all infected pheasants for several reasons. Antibiotics are widely used as feed additives on game farms in Iowa and may retard or suppress production of antibodies specific for avian Mycoplasma (Fahey and Crawley 1955). The length of induction period and the duration of infection relative to the collection of serum samples may influence the serological procedures.

ACKNOWLEDGEMENT

We wish to thank Dr. H. W. Yoder Jr. of the Iowa State Veterinary Medical Research Institute for his assistance, and the National Science Foundation for its Undergraduate Research Participant support for the senior author.

Literature Cited

Vertebrate Coactions With the Franklin Ground Squirrel

EMMETT POLDER

Abstract: Nearly 30 years of observation of Franklin ground squirrels in central and northeast Iowa indicates that they are of minor importance as prey for carnivorous birds, mammals, and reptiles. These ground squirrels are predators of some significance on eggs and young of ground-nesting birds and the helpless young of some small mammals. The Franklin squirrel burrows are of considerable importance as dens for long-tailed weasels and spotted skunks and are of seasonal value as retreats for young opossums, striped skunks, and mink during mid-summer dispersal. Amphibia use the dens as a moist daytime refuge during hot, dry weather and garter snakes sometimes use the burrows for winter hibernation. Damaged burrows are used by small rodents and insectivores and are utilized as nest sites by cottontails and ground-nesting birds.

INTRODUCTION

The Franklin ground squirrel (Citellus franklinii (Sabine) is probably present in every county of Iowa. It is locally abundant in colonies of from 10 to more than 100 individuals in favored habitats such as hay fields, oat fields, weedy fence rows, and native prairie. It is rarely found on timber soils or in woodlands. In central and northern Iowa the highest populations tend to concentrate on heavy moderately wet soils of glacial origin. The border zone between low wet soils formed under Spartina and soils formed under Andropogon appears to be the optimum habitat both on native prairie and on cultivated lands. In years when Franklin squirrels are at a high cyclic population level they are commonly seen on high well-drained ground and on loess soils where they are scarce or absent during years of low population.