Transmission and Eradication of Mycoplasma gallisepticum in Chickens

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PATHOGENIC avian mycoplasma are of great economic concern to the chicken and turkey industries (Anonymous, 1963; Yoder, 1965). Research endeavors have been very productive in broadening our knowledge concerning the various serotypes that have been identified (Adler, 1960; Edward and Kanarek, 1960; Kleckner, 1960; Yoder and Hofstad, 1964). The importance of egg transmission of the different serotypes in the perpetuation of avian mycoplasmalosis has been further elucidated (Adler et al., 1956; Fabricant et al., 1959; Olesiuk and Van Roekel, 1960; Van Roekel et al., 1952, 1958; Yamamoto et al., 1965; Yoder and Hofstad, 1964). The universal existence of these organisms among domestic poultry has been confirmed (Yoder, 1965). The serological tests have been improved and have served as useful tools in the diagnosis of the disease (Adler, 1954, 1958; Cover et al., 1960; Crawley and Fahey, 1957; Dierks, 1963; Hofstad, 1957; Jungherr et al., 1955). Attempts to control avian mycoplasmalosis through prophylactic and therapeutic antibiotic medication have mitigated the losses to a variable and limited degree (Adler et al., 1956; Crawley and Fahey, 1955; Dunlop and Strout, 1956; Jungherr, 1958; Ose et al., 1964; Olesiuk and Van Roekel, 1959; Olson et al., 1962; Peterson, 1965). Likewise accepted sanitary and management programs have only partially reduced losses from this disease (Anonymous, 1963, 1965; Chute and Stauffer, 1962; Jungherr, 1958; Olesiuk and Van Roekel, 1959; Olson et al., 1962; Peterson, 1965). More recently, control through immunization with a live virulent organism has been attempted on a large scale without causing any perceptible decrease in condemnation losses in broiler progeny from vaccinated parent stock (Anonymous, 1965; Yoder, 1965). A limited number of reports have revealed that a conscientious and determined effort to eradicate the disease from breeding flocks appears to be the most promising approach in eliminating the losses from M. gallisepticum (MG) infection (S-6 type) (Chute and O'Meara, 1963; Chute and Stauffer, 1963; Chute et al., 1964; Moulethrop, 1962; Olesiuk and Van Roekel, 1960; Peterson, 1963, 1964). Furthermore, once a flock has been established free of this infection its status can be maintained if adequate security measures against re-infection are observed. However, in the eradication of the disease one must be cognizant of its various modes of dissemination (Yoder, 1965).
In this paper observations concerning transmission of MG-infection and eradication results of this disease from chicken breeding flocks obtained over an 11-year period will be presented.

MATERIALS AND METHODS

Transmission experiments. The chickens used in the trials were obtained from known MG-infected and noninfected commercial breeding flocks. Also, a small MG-free breeding flock maintained at the laboratory provided a constant supply of hatching eggs and progeny. All MG-free chickens were maintained under isolated conditions. Laboratory-reared stock was immunized only against infectious bronchitis prior to experimental use, employing only laboratory-produced vaccines, prepared in MG-free eggs. Feed and bagasse (sugar cane) litter were obtained from commercial sources.

Culture media. Difco PPLO broth enriched with 10% sterile inactivated horse and swine sera was used for isolating and propagating the MG-organism. Five carbohydrates, (dextrose, maltose, lactose, sucrose and mannite) were used in a 1% solution in phenol red broth base plus 10% inactivated horse serum. For solid medium the PPLO broth plus 1% agar was used. Cultures collected from the nasal passages, sinuses, trachea, air sacs and egg yolk were placed in PPLO broth containing penicillin and thallium acetate as bacterial inhibitors. Seven-day-old embryonated eggs from MG-free sources were employed for the isolation and propagation of the organism. The inocula of the cultures or tissue suspensions were inoculated into the yolk sac. For solid medium the PPLO broth plus 1% agar was used. Cultures collected from the nasal passages, sinuses, trachea, air sacs and egg yolk were placed in PPLO broth containing penicillin and thallium acetate as bacterial inhibitors. Seven-day-old embryonated eggs from MG-free sources were employed for the isolation and propagation of the organism. The inocula of the cultures or tissue suspensions were inoculated into the yolk sac. The embryos were examined daily and appropriate harvests were made for further passage either into embryos, culture media or chickens. After 20 to 21 days of incubation all surviving embryos were examined for gross respiratory lesions. Occasionally, the embryos were permitted to hatch and the chicks were examined for lesions at one day of age.

Serologic tests. The rapid-serum-plate agglutination (RSPA), the tube agglutination (TA), and hemagglutination-inhibition (HI) tests were employed in these investigations. The procedures followed in conducting these tests were similar, with some modifications, to those described by other workers (Crawley and Fahey, 1957; Dierks, 1963; Fahey and Crawley, 1954; Jungherr et al., 1953). The MG-strain isolated from a turkey by Zander (1961) was used as the antigen strain. For the most part the antigens were produced in the laboratory although some, from other sources, were used primarily for comparative purposes.

Chicken inoculation tests. Cultures, embryo materials, and broth tissue suspensions were tested for infectivity in MG-free birds by intrasinus or/and intratracheal inoculation employing 0.5 ml of the inoculum per route. As a rule the inoculated birds were maintained in small isolation rooms. They were clinically observed for two or three weeks and then killed and examined for MG-lesions. Each bird was tested serologically at least twice for MG-antibodies by one or more methods. For artificial infection a virulent MG-strain (designated Hy) was employed. The origin of this strain has been described in a previous paper (Olesiuk and Van Roekel, 1960). This strain was propagated in embryonated eggs and maintained in harvested embryo-yolk at -30 to -50°F.

Eradication procedures in commercial flocks. Owners of several MG-infected primary breeding flocks volunteered to cooperate in an effort to eradicate the disease from their flocks. All flocks were not treated in the same manner and the procedures used to eradicate the infection may be enumerated as follows: special selection
of breeding birds (two years or older); antibiotic medication of the parent stock and progeny; rearing of the progeny in MG-free environment; periodic serologic testing of the parent stock and progeny; laboratory examination of embryos and cull chicks for respiratory lesions; clinical observations of the parent stock and progeny; planned immunization program for viral infections; and effective sanitary and security measures. Once a primary flock was considered free of MG-infection other commercial flocks derived from the primary flock were subjected to 100% flock test by the RSPA method. Also, most breeding flocks tested for pullorum disease were tested for MG-infection on a random basis and if no reactors were detected all birds on the premises were tested to determine the flock status. Questionable reactors or any other suspicious evidence of MG-infection were investigated. When birds gave questionable reactions they were either retested or, more frequently, submitted to the laboratory for further investigation. In some instances, depending upon circumstances within the flock, one or more pens of birds were retested. After the flocks had essentially completed their production year some were random tested to ascertain their MG-status.

RESULTS

TRANSMISSION STUDIES. A series of 11 transmission experiments were conducted during the past five years. A brief design and the results of the experiments are presented.

Experiment 1. Seventy-five MG-susceptible 5-week-old White Leghorn (WL) males were placed in each of two pens that had been occupied by artificially MG-infected stock 12 days previously. The infected birds, manifesting clinical respiratory signs, were held for four weeks and the used litter, feed and equipment were permitted to remain in the pens. In a clean pen, 105 WL males of the same age were used as controls. During an observation period of three months, seven bi-weekly serologic and cultural tests revealed no evidence of transmission. The mortality, attributed largely to leukosis in the groups was slight. The surviving birds were used for other purposes after the termination of the experiment.

Experiment 2. In this trial, 50 susceptible 10½-week-old WL birds (25 females and 25 males) were placed in a pen that had been occupied for four weeks by artificially MG-infected birds which were removed two days previously. Marked respiratory signs (rales) were observed in the inoculated birds. The used litter was permitted to remain in the pen but clean water fountains and feeders were provided. Forty-three controls of a similar age were maintained in a separate building. Six birds in each group died from either coccidiosis or leukosis during the course of the experiment. The trial was terminated at 59 days and no evidence of transmission was detected by serology, clinical signs, and gross pathology. The control birds also remained negative.

Experiment 3. Thirty-eight susceptible mature chickens (30 WL females, 6 WL males and 2 Rhode Island Red (RIR) males) were placed in cohabitation with 43 naturally infected, asymptomatic, serologically positive sex link (SL) hens. These yearling hens had undergone a natural outbreak at about two months of age. Twenty 8-week-old susceptible WL males were maintained in a four-tier battery that had been placed in the pen. No evidence of transmission was noted in either group during the 16-week trial. A total of ten birds died from leucosis or other causes during the course of the experiment; two from positive group, two susceptible battery birds, and six susceptible pen birds. Hatch-
### Table 1.—Mycoplasma gallisepticum transmission studies from artificially infected to susceptible chickens

<table>
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<tr>
<th>Groups</th>
<th>No. Birds</th>
<th>Method of Exposure</th>
<th>Holding Period (days)</th>
<th>Mortality</th>
<th>Serology 28 da.</th>
<th>No. with Signs</th>
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<th>Birds with Airsacculitis</th>
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* Pens A and B are adjoining pens separated by a partition (upper part wire) and separated from Pen C by a central hall; Pen D in a separate building; Isolated Rooms 1, 2, and 3 are separate in another building. Birds in groups 1, 2, and 3 were moved to new quarters on the 28th or 29th day of the experiment.

* A total of 75 birds from groups 1, 3, and 8 was retained for other purposes; 44, 14, and 17 respectively.

* Involvement of posterior thoracic, lesser, and greater abdominal air sacs scored for each bird. S = One or more air sacs slightly involved. M = One or more air sacs markedly involved.

* Numerator equals number positive; denominator equals number tested.

* These birds sacrificed on the 28th day of experiment for gross pathological examination. Inoculum—Hy strain; route—intratracheal at onset; intratracheal and intrasinus at 14th day; dose—0.5 ml/route.
fested airsacculitis. Birds in group 2, in contact with group 1 for the first 28 days of the experiment, revealed one reactor but no signs on the 28th day. On the 42nd day the majority were serologically positive and six manifested signs. On the 56th day the number of reactors had increased slightly and the signs had decreased. At the termination of the experiment seven birds revealed airsacculitis. The birds (group 5) placed in direct contact with group 1 on the 28th day, also revealed evidence of transmission but not to the extent as observed in group 2. At the termination of the experiment, birds of groups 1 and 6 manifested respiratory signs and on necropsy, the majority of birds revealed a slight airsacculitis. No evidence of transmission was observed in the remaining groups of birds. These results suggest that under certain circumstances transmission through cohabitation with infected birds may occur readily; whereas, with indirect contact, spread was not detected.

Experiment 5. Twenty naturally infected seven-month-old White Plymouth Rock (WR) female chickens were placed in cohabitation with 20 MG-free BR females (ten months of age) in a pen simulating practical field conditions. The WR females had experienced clinical signs of MG-infection at 12 weeks of age on a commercial farm and were obtained at sexual maturity for laboratory study. All birds were serologically positive and asymptomatic at the start of the experiment. The BR stock was purchased from a MG-free commercial flock as ready-to-lay pullets. No evidence of transmission was detected by serology, culture of fresh eggs, signs, and gross lesions during 23 weeks of direct contact. The five inoculated birds revealed no lesions at necropsy, tissues from the turbinates and trachea were harvested and used for embryo and bird inoculation. The five inoculated birds remained asymptomatic but all five revealed slight gross respiratory lesions and three became serologically positive. Tissues harvested from these inoculated birds failed to induce a serologic response or significant gross lesions when further passed in birds. Embryonated eggs inoculated with the same suspensions revealed air sac lesions at hatching time.

Experiment 6. Two groups of birds designated A and B were each maintained in different buildings. Group A consisted of 77 eight-month-old WR birds (69 females and 8 males) and 20 ten-month-old BR females. Group B consisted of 12 WR birds (10 females and 2 males) and 10 BR females. The 12 WR birds in this group were selected on the basis of a strong serological reaction. Positive and susceptible birds for
groups A and B were obtained from the same sources as described in experiment 5. 

The birds in Group A were in direct contact for 20 weeks and conclusive evidence of transmission did not appear until about the 16th week of contact and within two weeks all the susceptible BR hens became serologically positive. Tracheal rales were detected in seven birds during this two-week period. Previously, on the 11th week of the experiment the WR males were sacrificed and replaced with ten susceptible WL males (11 months old) in order to improve the fertility in the hatching eggs. The WR males were positive serologically but no gross pathological evidence of MG-infection was detected. After a period of two weeks the WL males were also removed and sacrificed because of poor egg fertility. The birds revealed no serological evidence or gross pathology of MG-infection. Seven negative BR males (13 months old) were added to the flock which was two weeks prior to the time evidence of transmission was first detected. These BR males all became serologically positive five weeks after their introduction into the group. During weekly testing of the BR hens it was observed that one bird gave a 4+ reaction on the 5th week, 3+ on the 6th week, 1+ on the 7th week and then remained serologically negative until the group became positive 9 weeks later. Also, progeny hatched from the BR dams exhibited parental agglutinins after the group became positive. Agglutinins were detected in progeny of 13 dams during the terminal four weeks of the experiment. Only occasional respiratory lesions were detected in the day-old progeny of WR and BR dams. When the experiment was terminated the WR and BR birds were used in a subsequent indirect transmission trial (experiment 7).

The birds in Group B were in direct contact for 17 weeks. About 11 weeks after the experiment was initiated one male died and the other was destroyed. These were replaced with 11-month-old susceptible WL males for approximately two weeks. After that time the flock was not mated. No evidence of MG-transmission was observed in the BR hens. One WR and one BR hen died during the contact period. Nine BR hens were challenged with the Hy strain. Accidentally five birds died from heat stroke while on challenge. The four surviving birds manifested clinical signs and positive serology for MG-infection. These birds were used in subsequent indirect transmission trials. The nine surviving WR females were killed and their respiratory tissues pooled and inoculated into susceptible birds and embryonated eggs. Gross lesions and MG-agglutinins were detected in the inoculated birds. Likewise, chicks hatched from inoculated embryos manifested air sac lesions. Progeny hatched from the WR group, while mated, revealed agglutinins and occasional respiratory lesions.

Experiment 7. An attempt to demonstrate indirect transmission by exchanging four paired groups of infected and susceptible birds in their respective pens on a bi-weekly schedule for six rotations was made. The same litter and feeding equipment was used for the duration (14 weeks) of the experiment. Two naturally infected and two artificially infected groups were used while laboratory-reared 11-week-old BR and 10-week-old WL males composed the susceptible groups.

Group 1 consisted of 62 naturally infected WR hens 13 months of age that had experienced a respiratory disease outbreak of MG-infection at 12 weeks of age. All birds were asymptomatic and reacted positively with MG-antigen. These hens were used in Experiment 6 and were retained for this experiment. Twenty BR males represented the paired susceptible group.

Group 2 consisted of 19 BR hens and 3
BR males that had been used in a previous exposure trial with naturally infected hens (Experiment 6). The BR hens and males had become serologically positive and manifested clinical signs during the four previous weeks to the current trial. The paired susceptible group consisted of 20 BR males.

Group 3 was composed of 25 ten-week-old WL males that were artificially inoculated with yolk suspension of the Hy strain. Fourteen days postinoculation 13 birds exhibited rales and essentially all were serologically positive. Twenty-five WL males from the same source represented the paired susceptible group.

Group 4 consisted of 26 BR hens (15 months of age) that failed to contract infection when exposed by direct contact to naturally infected stock. The hens were artificially inoculated following the same procedure as used in Group 3. Also, four serologically positive BR males that were mates to the positive males in Group 2 were added to the positive hens. Twenty 10-week-old WL males composed the paired susceptible group.

Periodic serologic tests revealed no change in the serologic status of the positive groups. In Groups 2, 3 and 4 the birds in the positive groups manifested clinical respiratory signs during the early part of the trial. No serologic response or signs were observed among the susceptible groups with the exception of Group 1. In this group three birds gave doubtful reactions on one or two tests but later they were negative. Among the susceptible birds in Groups 1 and 2 mortality that was attributed to leukosis was observed in seven birds in each group. At approximately 14 weeks the trial was terminated and all the birds were killed and examined. Seven birds in the positive groups exhibited suspicious residual gross lesions in the respiratory tract. Among the surviving susceptible birds three revealed questionable lesions in the air sacs. Nine broth pooled suspensions of nasal and tracheal tissues were prepared from the sixty positive hens in Group 1 and each pooled suspension was inoculated into five susceptible chickens via the sinus and tracheal routes. Also, each suspension was inoculated into embryonated eggs and into PPLO broth. The positive birds in Groups 2, 3 and 4 were treated similarly as those in Group 1. The suspensions from the different groups of birds yielded positive evidence of MG-infection by culture isolation and by embryo and bird inoculations. Since no transmission was evident, based on the absence of clinical signs, gross lesions and serological response, tissues from the respiratory tract were not collected from the birds in the susceptible groups. While no evidence of indirect transmission through used litter and equipment was observed, it was established that at least some birds in each positive group at the end of the trial were still harboring *M. gallisepticum*.

Experiment 8. Eighteen asymptomatic, serologically positive SL hens (16 months of age) were placed in cohabitation with 92 susceptible WL hens (15 months of age). The SL birds, obtained from a commercial egg-laying flock, first manifested MG-infection at three months of age. The hens were not mated and were maintained in a conventional type poultry house. The duration of the experiment was 65 days. One SL and three WL hens died from miscellaneous diseases during the trial. The positive hens maintained their positive serologic status but exhibited no clinical respiratory signs while under observation. The susceptible group first revealed serologically reacting birds on day 23 of the experiment. Nine birds, giving various degrees of reaction, were detected in the total weekly tests. However, some birds reacted only on one or two tests. Two birds gave a strong reac-
tion on five or more successive tests. In the last test one suspicious and three strong reactors were detected. Only the serologically suspicious and positive WL hens and the SL hen were necropsied. The latter revealed no clinical signs or gross pathological evidence of MG-infection, whereas, two WL hens exhibited rales and gross respiratory lesions. Since the purpose of the experiment was to demonstrate transmission of the disease it was decided that the trial be terminated as soon as evidence of infection was detected in the susceptible birds.

Experiment 9. Twenty serologically positive WR females (9 months old) and 20 susceptible BR females (7 months old) were placed in cohabitation in quarters simulating commercial conditions. The WR group suffered an outbreak of the disease at an early age and at 6 months of age the rate of serologic reactors approached 100%. During a 23-week contact period one WR bird manifested rales at 15 weeks. At the termination of the trial, 19 of the 20 WR birds remained serologically positive and one revealed suspicious air sac lesions. *M. gallisepticum* was isolated from respiratory tract suspensions from six of the 20 WR birds cultured, three on embryo inoculation, one in broth, and two using both methods. In the BR group seven birds revealed respiratory rales, and 11 birds manifested both positive serological and gross pathological evidence of MG-infection. The first evidence of transmission was noted 71 days after initial contact between the two groups. One BR manifested rales and was strongly positive serologically. Six weeks elapsed before there was evidence of further spread. Suspensions prepared from the respiratory tract of the 18 BR birds and inoculated into PPLO broth and embryo-nated eggs yielded positive isolations from nine birds, three in broth, five on embryo inoculation, and one using both methods. Pedigree fresh eggs (1167 WR and 1419 BR) cultured during the trial yielded negative results for MG-infection. Two BR females died during the early period of the trial.

The 20 WR females mentioned above were also simultaneously used for a transmission experiment using their feces and force-feeding them to susceptible chickens. The positive birds were placed in a wire battery overnight in groups of five birds per tier. The feces from each group were pooled and suspended in broth. Each suspension was force-fed to 14 susceptible chickens (ten 8-week cockerels and 4 mature hens) and each group received two feedings weekly for four weeks of fecal suspensions prepared from the respective groups of positive birds. Four weeks after the first feeding serological reactions were detected in two groups. At the end of eight weeks all forced-fed birds were killed and no gross lesions were detected. No signs were noted but agglutinins did persist in some birds from these two groups during the last four weeks of the trial. Unfortunately, the trial was terminated too soon to ascertain whether transmission of the infection had actually occurred.

Experiment 10. Thirty-one MG-positive, WR females were placed in cohabitation with 24 susceptible BR birds (20 females and 4 males) in a pen simulating commercial husbandry conditions. Eleven of the WR females were introduced 21 weeks after the trial was initiated. The source and age of the birds were the same as in experiment 9. No evidence of transmission was noted after 36 weeks of contact. Seven females and one male in the BR group died from various causes and no gross lesions were detected in the remaining birds. The WR females remained serologically positive and were used in experiment 11. In 18 pedigree hatches (April through July) 1139 WR and 1088 BR progeny were hatched and none revealed gross re-
spiratory lesions. Among the WR progeny 577 revealed parental agglutinins whereas the BR chicks were negative.

**Experiment 11.** Two groups of chickens designated A and B were each maintained in a separate house that simulated field conditions. Fifteen serologically positive, 23-month-old WR hens, retained from experiment 10, and 12 susceptible 10-month-old BR birds (10 hens and 2 males) were placed in each of two houses. The positive birds were obtained from a commercial flock at approximately six months of age after they were found to be serologically positive for MG-infection. Clinical or serological evidence of transmission was not observed among the birds in either house during a period of 14 weeks. Two of the 15 WR hens in group A became negative serologically while all the WR birds in group B continued positive.

In group A one BR female died, and the two BR males were killed four weeks after the trial was started and replaced with MG-free WL males. After 14 weeks, nine BR hens and five WR hens were challenged with the Hy strain. Typical signs, lesions and serology for CRD were observed in the BR females but no signs or lesions were seen in the WR hens. The remaining ten WR birds were necropsied and two pools of tissue, collected from the respiratory tract were prepared. Susceptible birds inoculated with these tissue suspensions yielded erratic results while embryo inoculation and isolation in PPLO broth gave evidence of MG-infection.

In group B the BR males were also destroyed four weeks after the trial was started and replaced with two WL males free of MG-infection. The challenge results of the ten BR hens were similar to those in trial A. Three pools of respiratory tissue obtained from the WR hens and inoculated into susceptible chickens yielded erratic results even after three bird passages. The respiratory tissue suspensions when subjected to embryo inoculation and isolation in PPLO broth yielded positive results.
at the laboratory by serological testing and bird inoculation tests they were found to be negative. From a total of 924 embryos and day-old chicks examined from this flock, 20 specimens revealed only very questionable air sac lesions for MG-infection. During the ten successive years, this flock was tested on a 100% basis at pullorum testing time when the birds were five months or older in age. A brief summary of the testing results and complementary findings are presented in Table 2. It is evident from the results that the existence of MG-infection in the flock was never unequivocally established in a manner or to a degree that was commonly observed in chicken breeding flocks. The limited and transient suspicious serological reactions that were encountered are regarded as atypical or non-specific for the S-6 type infection. However, tracheal swabs immersed in PPLO broth and the suspensions inoculated into embryonated eggs produced gross respiratory lesions in embryos that resembled the lesions produced by the S-6 type infection. Attempts to isolate and propagate an S-6 type were unsuccessful. A few doubtful reactors appeared in this flock in 7 of the 11 years. Random blood samples, collected from the flock five or more months after the total test, revealed no reactors. Embryos and cull chicks received from the hatchery in seven different years were examined and suspicious lesions were detected among the specimens representing five of those years. In evaluating the MG-status of the flock, in view of the fact that unequivocal evidence of the disease was not established, the flock was classified as free of the infection. This free status is further substantiated by the negative serological evidence obtained in other breeding and multiplier
Table 3.—Nine year summary of 16 multiplier chicken flocks that originated from one primary breeding flock free of *M. gallisepticum*

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<td>1960</td>
<td>10</td>
<td>38,754</td>
<td>4 2 4 33 4 2 3 2 2 2 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1961</td>
<td>11</td>
<td>57,329</td>
<td>10 1 0 0 0 0 1 2 4 1 2 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1962</td>
<td>9</td>
<td>46,602</td>
<td>6 0 3 8 2 1 1 1 3 1 2 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1963</td>
<td>11</td>
<td>66,247</td>
<td>11 0 0 0 0 0 4 1 3 1 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1964</td>
<td>9</td>
<td>78,338</td>
<td>6 0 3 18 3 0 2 3 1 2 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on 5-month rapid serum plate agglutination test.

b Based on additional laboratory tests of reactors and flock tests.

c Flock positive by random test, 294 positive of 2476 tested.

d Flock #1, 532 positive of 1004 tested (random test).

Flock #2, 213 positive of 4265 tested (random test).

e Flock positive by random test, 296 positive of 306 tested.

f Flocks that were progeny of flock A.

Table 3 gives a testing summary of the progeny flocks from flock A located on 16 premises that were 100% or in a few cases random tested, one or more years from 1956 to 1964, inclusive. In most instances the flocks were started as day-old-chicks but in some started pullets were purchased. The size of the flocks ranged from less than a thousand to 17 thousand birds. Most of the flocks were reared on range until sexual maturity. All flocks were immunized against the common avian viral diseases with live vaccines. A total of 62 flocks representing 330,113 birds was tested during the nine-year period. In eight of the nine years, 20 flocks revealed a total of 1410 reacting birds. Seventy-three birds from 16 doubtful reactor-flocks were submitted to the laboratory for further study. The remaining four flocks, representing two different premises, one tested in 1959 and 1960 and the second in 1960 and 1961, yielded 1335 reactors among 10,752 birds tested and were classified as MG-positive without additional tests. The owner of the first premises had raised stock from infected sources prior to 1959 and his stock did not become negative until 1961. The second owner also raised stock from infected sources prior to 1960 and this operation did not establish a negative flock until 1963. In 1962 the second operation, revealing 6 doubtful reactors in a group of 6547 birds, was classified as positive on findings of additional laboratory tests. One additional flock, tested in 1957, was classified as positive for MG-infection based on findings of follow-up tests.

Fourteen of the 16 doubtful reactor-flocks were classified as negative based on additional laboratory tests of reactors and flock tests. In 1956, the three doubtful reactors of the single flock tested were negative by follow-up tests at the laboratory. The two doubtful reactor-flocks detected in 1958 were classified as negative based on findings of additional tests. In 1960 the results on three of the four reactor flocks were difficult to interpret. In one flock, 12 reacting birds were examined, five of which became serologically negative and the remaining seven gave a weak reaction after a period of four weeks. Cultural and embryo
inoculation results gave a suspicion of infection but bird inoculations with the same suspensions were negative. In the second flock, the only evidence suggestive of infection was obtained in inoculated embryos. However, random serological test of the flock some months later revealed no reactors. The third flock revealed results similar to the second flock. It was also random tested some months later and no reactors were detected. Follow-up studies in the fourth flock were negative. In 1962 three reacting flocks were detected; two with one reactor each which yielded negative results at the laboratory, and a third with six reactors was classified as positive for MG-infection. In 1964 three doubtful reactor-flocks, with 1, 5 and 12 reactors, were classified as negative by additional tests. During this nine-year testing period, some owners of these flocks either discontinued poultry operations or purchased birds from other sources that were infected. However, the data reveals that some owners were successful in maintaining a negative flock for as long as seven consecutive years. These results show conclusively that flocks free of MG-infection can be established and maintained if free stock is obtained and reared in an environment free of this infection.

Flocks B and C. An attempt was made on two primary breeding farms to establish MG-free progeny from infected dams. The approach was one of early treatment of chicks with tylosin in an effort to break the infection cycle that prevailed in these two flocks. Reasonable sanitary precautions were established on both farms.

In flock B the infection rate fluctuated from year to year and was not always extensively established in the flock as is commonly seen in most infected flocks. The reactor rate in the pedigree hens (one year or older) was 212 among 338 tested (Table 4). Eight pedigree hatches were obtained from these hens from January to June 1963. The first hatch was given two tylosin subcutaneous injections (60 mg./bird), one at three months and the second three weeks later. The chicks of the remaining hatches were placed on the following medication schedule: water medication (tylosin 4g./gal.) at one week of age for five days; two subcutaneous injections of tylosin, one at four weeks (25 mg./bird); and one at eight weeks, (37.5 mg./bird). This medication regimen was followed a second year on the pedigree replacements for the flock. The chicks were brooded in conventional type brooding facilities and later the majority of the birds were reared on isolated grass ranges. The chicks were serologically tested at three and five months of age. The physical facilities for this flock consisted of two separate farms and also two other farms that were rented during the brooding and rearing periods. These facilities permitted the installation of isolation measures.

In flock C, eight pedigree hatches were obtained from hens, one year or older, during April through June 1963. The medication schedule was similar to that of flock B excepting that the dams were given two tylosin injections (75 and 90 mg./bird) prior to and during the time that eggs were collected for the eight hatches. A total of 360 dams was treated which revealed a 100% reactor rate (Table 4). The above program was repeated a second year in this flock.

No reactors were detected in the progeny of flock B that was treated in 1963. In 1964 the replacements were produced from MG-free breeders (hatched 1963). In the summer of 1964 all positive adult stock on the farm was sold. In 1965 the replacement stock was produced from both pullets reared in 1964 and one-year-old hens.
Table 4. *M. gallisepticum* eradication results in flocks B and C

<table>
<thead>
<tr>
<th>Testing Year</th>
<th>Flock Size</th>
<th>Pedigree breeding dams</th>
<th>Examination results</th>
<th>Progeny serology</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>% React.</td>
<td>Lesions</td>
<td>Lesions</td>
</tr>
<tr>
<td>1963</td>
<td>14,005</td>
<td>338</td>
<td>63</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>17,406</td>
<td>379</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1965</td>
<td>12,656</td>
<td>942a</td>
<td>0</td>
<td>NR</td>
</tr>
<tr>
<td>1963</td>
<td>5,254</td>
<td>360</td>
<td>100</td>
<td>8</td>
<td>1/219</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>6,298</td>
<td>360</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1965</td>
<td>5,667</td>
<td>350</td>
<td>100</td>
<td>NR</td>
</tr>
</tbody>
</table>

* Flock size = total adult birds for yearly pullorum testing period ending March 31.

b Number positive/number tested.
a Breeders in pedigree pens and penmated birds used to produce flock replacements.
d An additional 514 birds on experiment not tested because of definite CRD break.


Reared in 1963. The progeny produced in 1965 revealed eight suspicious reactors which were negative on laboratory examination. The owner was impressed by the improved performance of his flock since *M. gallisepticum* was eliminated.

The results in flock C were slightly less successful than in flock B. All of the infection was not eliminated during the first year that the progeny and dams were treated. However, during 1964 the owner was successful in eliminating the infection which was validated by the results obtained in 1965. The physical facilities for flock C were not as adequate as for flock B. Also, the initial reactor rate was higher in flock C than in B. The owner of flock C was also impressed by the improved performance of the flock after the infection was eliminated.

**Flock D.** This primary breeding flock of BR, RIR, and WR stock has been tested for MG-infection for nine consecutive years, 1957–1965. Table 5 reveals that no stock was introduced from other sources in the last five years. Random samples collected from the flock during the first five years revealed a high reactor rate, especially among the hens. In 1961, the reactor rates were 117 and 196 among 8067 pullets and 269 hens, respectively. In 1962 a random test of the hens and WR pullets revealed a high reactor rate while a total test of the BR and RIR pullets and males was negative. In 1963, the entire flock was tested and all but five of the 153 reactors were confined to the hens. In 1964, a 100% test of the flock revealed only one reactor, a BR hen. Between the 1963 and 1964 testing years the owner disposed of all the in-
TABLE 5.—A nine-year testing summary for M. gallisepticum infection in primary breeding flock D

<table>
<thead>
<tr>
<th>Testing Year</th>
<th>Flock Size</th>
<th>RSPA Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Random Test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>1957</td>
<td>10,137</td>
<td>140P</td>
</tr>
<tr>
<td>1958</td>
<td>11,816</td>
<td>60P</td>
</tr>
<tr>
<td>1959</td>
<td>10,683</td>
<td>55P</td>
</tr>
<tr>
<td>1960</td>
<td>11,166</td>
<td>85P</td>
</tr>
<tr>
<td>1961</td>
<td>10,965</td>
<td>8067P</td>
</tr>
<tr>
<td>1962</td>
<td>11,051</td>
<td>158H</td>
</tr>
<tr>
<td>1963</td>
<td>10,475</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7805P</td>
</tr>
<tr>
<td>1964</td>
<td>8,956</td>
<td>1112H</td>
</tr>
<tr>
<td></td>
<td></td>
<td>426M</td>
</tr>
<tr>
<td>1965</td>
<td>9,181</td>
<td>2762H</td>
</tr>
<tr>
<td></td>
<td></td>
<td>353M</td>
</tr>
</tbody>
</table>

Remarks
- Introduced stock from infected and free flocks.
- Introduced stock from known infected source.
- No stock introduced.
- Introduced stock from an infected source.
- No stock introduced during 1961 to 1965 testing years.

Remarks
- Hens = BR, RIR and WR stock. Reactors detected in RIR pullets and males. No follow-up tests made.
- Follow-up serology, embryo and bird inoculation results suspicious of MG. Random test of 210 birds 5 mo. later revealed no reactors.
- Follow-up serology and cultures —neg. Random test of 210 birds 6 mo. later revealed no reactors.

H = hens; P = pullets; M = males

* Only 60 birds tested in a group of 219 WR pullets.
* b Represent only BR and RIR pullets.
* c Included 180 WR males.
* d Reactor was a BR hen.
* e SL hens negative previous year.
* f Two RIR reactors detected in a group of 3955 RIR pullets.

A nine-year testing summary for M. gallisepticum infection in primary breeding flock D. Testing beginning in 1957 and continuing through 1965, the year of observation. Testing was done on a random basis, with birds selected from various groups of the flock for test purposes. After each testing year, the flock was separated into groups, two groups being inoculated with tracheal suspensions of M. gallisepticum. The remaining groups were tested with the RSPA method over a five-month period. However, negative results were obtained with the TA and HI methods. Four separate groups of chickens were inoculated with tracheal suspensions which failed to produce positive evidence of infection. On the contrary, inoculated embryos developed marked air sac lesions. Whether these lesions were produced by an S-6 type avian mycoplasma was not confirmed. However, it appears that the flock was negative for the S-6 type since no reactors were detected by a random test conducted in the spring of 1965. In the 1965 testing season, two reactors were detected among 1694 SL hens that were negative the previous year. Also, two reactors were detected among 3955 RIR pullets. All four reactors were submitted for further examination but due to some misfortune at the laboratory only a limited investigation was made, the results of which proved to be negative.
TABLE 6.—M. gallisepticum testing results of supply flocks for a single hatchery during a five-year period

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Flocks</th>
<th>Number of Birds</th>
<th>Classification of flocks</th>
<th>Reclassification of doubtful flocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>1961</td>
<td>18</td>
<td>18,222</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>1962</td>
<td>29</td>
<td>44,603</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>1963</td>
<td>26</td>
<td>48,572</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>1964</td>
<td>18</td>
<td>22,447</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>1965</td>
<td>10</td>
<td>20,985</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

a Results of 5-month total flock test.
b Results based on additional laboratory tests of reactors or additional flock tests of doubtful flocks.
° Additional tests in these flocks revealed a low level of infection.

**Eradication of M. gallisepticum from supply flocks for a single hatchery.** In cooperation with a large breeding corporation, assistance was given in a carefully designed program to replace MG-infected breeding flocks with MG-free stock to be used as supply flocks for a local hatchery. A period of 12 months was allowed for replacement of the infected flocks with negative stock. The premises for brooding and rearing the negative birds were carefully selected and special sanitary precautions were taken. At the local hatchery isolated incubation, hatching and cleaning facilities and special caretakers were provided to hatch the negative stock. Hatching eggs from known MG-free breeding birds were supplied to the hatchery. This WR breeding operation was described earlier by Olesiuk and Van Roekel (1960). The replacement flocks were carefully inspected at periodic intervals and immunized for infectious bronchitis and Newcastle disease. The flocks were serologically tested on a total basis at 10 to 12 weeks of age and at five months when the birds were also tested for pullorum disease. At 14 months a random flock test was conducted prior to disposition of the flocks.

Table 6 gives a brief summary of the eradication results for a five-year period. In 1961 one doubtful reactor in one flock was detected which proved to be negative on further tests. In 1962 a total of 29 flocks was tested, of which one revealed definite infection and four yielded doubtful reactors on testing at five months of age. These doubtful flocks were reclassified as negative based on additional tests. Eighteen of the 28 negative flocks were tested on a random basis at approximately 14 months of age with negative results. In 1963 twenty-six flocks were tested, of which two were definitely positive, six doubtful, and 18 negative. Four of the six doubtful flocks were reclassified as negative by additional tests. Two flocks continued to reveal a low percentage of reactors until 14 months of age at which time the flocks were disbanded. Thirteen of the 18 negative flocks were random tested at 14 months of age and two revealed 50% or more reactors. Pasteurellosis had also been a problem on these four positive premises and all four farms belonged to the same owner. These four flocks appeared to have acquired the MG-infection. In 1964 16 of the 18 flocks tested were classified as negative at the five-month test. Thirteen of the 18 negative flocks were random tested at 14 months of age and two revealed 50% or more reactors. Pasteurellosis had also been a problem on these four positive premises and all four farms belonged to the same owner. These four flocks appeared to have acquired the MG-infection. In 1964 16 of the 18 flocks tested were classified as negative at the five-month test. Three doubtful reactors were detected among two flocks which proved to be negative on further testing. However, at 14 months of age six of 16 flocks tested revealed a low percentage of reacting birds. One of these flocks had been classified as doubtful at the five-month test. These reactions were not typical and had not been ob-
TABLE 7.—Mycoplasma gallisepticum negative breeding flocks classified as to consecutive years negative—1958-1964

<table>
<thead>
<tr>
<th>Year</th>
<th>Number Flocks</th>
<th>Number Birds</th>
<th>Intermittent</th>
<th>Consecutive years negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1958b</td>
<td>16</td>
<td>43,264</td>
<td>1</td>
<td>1 2 3 4 5 6 7 8 9</td>
</tr>
<tr>
<td>1959</td>
<td>17</td>
<td>51,071</td>
<td>0</td>
<td>0 2 4 5 6 7 8 9</td>
</tr>
<tr>
<td>1960</td>
<td>18</td>
<td>69,302</td>
<td>1</td>
<td>1 2 4 5 6 7 8 9</td>
</tr>
<tr>
<td>1961</td>
<td>27</td>
<td>108,355</td>
<td>1</td>
<td>1 2 4 5 6 7 8 9</td>
</tr>
<tr>
<td>1962</td>
<td>22</td>
<td>88,112</td>
<td>1</td>
<td>1 2 4 5 6 7 8 9</td>
</tr>
<tr>
<td>1963</td>
<td>31</td>
<td>124,861</td>
<td>3</td>
<td>1 2 4 5 6 7 8 9</td>
</tr>
<tr>
<td>1964</td>
<td>38</td>
<td>191,412</td>
<td>1</td>
<td>1 2 4 5 6 7 8 9</td>
</tr>
</tbody>
</table>

* Intermittent = flocks that had been alternately negative and positive.

† 1958 negative flocks checked for CRD status for the years 1956 and 1957.

served previously in flocks that tested negative at five months of age. These flocks originated from two sources of parent stock, three from each source. In 1965 ten flocks were tested which revealed 53 reacting birds among seven flocks. The presence of infection could not be confirmed in any of these flocks by additional tests. No reactors were detected in these seven flocks on the 14-month random test.

The source of the breaks in the above flocks could not be definitely ascertained. In the last two of the five years a relaxation in security measures was observed. In general, however, these results are most encouraging since it appears that it may be feasible to establish a MG-negative hatchery. The follow-up of reactors and the adoption of effective security measures cannot be stressed too strongly.

Nine-year summary of M. gallisepticum negative breeding flocks. Since the RSPA test for MG-infection was introduced, an effort was made to test as many breeding flocks as possible to determine their disease status and to use this information to ascertain if eradication of the disease was feasible. Since the state pullorum testing program was carried on in the same department, the blood samples tested for pullorum disease were also tested for MG-infection. Essentially all the antigens used for the serological tests were prepared in the laboratory. All flocks to qualify as negative were 100% tested and revealed no evidence of infection. Table 7 lists the number of negative flocks, number of birds tested, and the number of consecutive years negative for each year from 1958 to 1964. During this period the increases for flocks and birds were from 16 to 38 and from 43,264 to 191,412, respectively. In 1964, 14 flocks had been negative for four or more consecutive years. The number of MG-negative birds in 1964 was approximately one-third of the number of breeding birds tested for pullorum disease. As the infection is eliminated from additional primary breeding flocks the eradication of the disease will progress more rapidly. These results reveal conclusively that M. gallisepticum is amenable to eradication and that free flocks can be maintained as negative if proper precautions are taken.

Reactor rates in naturally injected flocks. Among breeding flocks tested for pullorum disease an appreciable number were tested on a random sample basis for MG-infection. In many instances the flock histories revealed the source or sources of the stock in the flocks. Table 8 shows that over a five-year period, 221 flocks were tested. One-third of the flocks revealed 24% or less reactors among the random samples tested. Sixty-three percent of the flocks revealed 50% or more reactors. Forty-six
percent of the flocks revealed a reactor rate from 75–100%. The pattern of distribution of reactor rates is very similar for the different years. However, one should recognize that these reactor rates may not represent the actual status of the entire flock since in the majority of flocks the reactor rate is based on a small random sample. It may be stated that in some flocks with a low reactor rate this rate did not change over a period of months or during the productive year of the flock. In flocks in which the level of infection is low and remains constant, the stock can frequently be successfully replaced with birds from free sources, completely eliminating the infection from the premises. On the other hand, a low reactor rate gives no assurance that it will remain at that level, since some flocks have experienced a marked increase in reactors or even a clinical outbreak of the disease. This is more apt to occur if stock from infected breeding flocks is introduced. This is illustrated in flocks E, F and H in Table 9. Flock A had a low flock reactor rate for a number of years. However, when the rates were calculated by age groups of birds on the premises, the pattern was entirely different. This operator maintained two to five hundred yearling birds each year. These birds showed a reactor rate in the range of 70 to 95% over the past four years. In 1965, the young birds experienced a natural outbreak shortly after the MG-test was conducted. The infection has thus been perpetuated in the flock in this manner. Flock B is the same flock as referred to in Table 5 which suggests that by not introducing infected stock the residual infection may submit to eradication through proper management. Flocks C, D and G maintained a relatively constant level of infection.

### Influence of antibiotic therapy on M. gallisepticum serology.

A naturally MG-infected group of 40 hens and 6 males were given 6 weekly subcutaneous injections of tylosin at the rate of 12.5 mg./lb. of body weight. A group of 20 females and 3 males from the same source as the treated group served as controls. Serological results revealed that the number of reactors did not change over a period of 12 weeks (Table 10). Also, progeny hatched from both groups revealed no significant changes in serological pattern before and after the dams were treated. It appears that once a
serologic titer becomes established in a bird, it cannot be altered by antibiotic medication. However, this does not apply when a flock is experiencing a symptomatic or asymptomatic outbreak of the disease (Adler, 1958).

DISCUSSION

Various aspects concerning the modes of transmission of *M. gallisepticum* have been reported and discussed by numerous workers (Adler et al., 1956; Fahey and Crawley, 1955; Grumbles et al., 1952; Jungherr, 1958; Lancaster et al., 1960; Olesiuk and Van Roekel, 1960; Van Roekel and Olesiuk, 1952, Yoder and Hofstad, 1964). The egg-borne route is still regarded as the most common means of spread for the disease (Calnek and Levine, 1957; Fabricant et al., 1959; Fahey and Crawley, 1954a; Jungherr et al., 1955; Van Roekel et al., 1952, 1958; Yamamoto et al., 1965; Yoder and Hofstad, 1964). The role of the infected host spreading the disease to susceptible birds cannot be minimized (Grumbles et al., 1952; Jungherr, 1958; Jungherr et al., 1955; Lancaster et al., 1960; Olesiuk and Van Roekel, 1960; Yoder, 1965). The results obtained in these investigations further elucidate our knowledge concerning this phase of the disease.

In seven of eleven transmission experiments concerning MG-infection, naturally infected chickens were used and transmission through cohabitation occurred in only three instances. In experiment 6 the first evidence of transmission was detected after 16 weeks of contact while in experiments 8 and 9 evidence was first detected at 23 days and 10 weeks, respectively. In the latter case, after the first infected bird was noted, no further spread was observed for 6 weeks but from then on, the rate increased rapidly. Also in experiment 9, one bird of the positive group manifested tracheal rales at about the same time rales were detected in the first of the susceptible birds. While rales may not always be consistently detected in birds with a mild tracheitis it seems plausible that such birds may shed the organism more readily than serologically positive birds without detectable signs or gross pathology. It is apparent that transmission may occur readily when birds with frank clinical signs of the disease exist in the flock. This has been readily demonstrated through cohabitation of inoculated and uninoculated birds as was the case in experiment 4. On the contrary, serologically positive flocks that do not manifest signs of the disease may also be capable of disseminating the disease, as was demonstrated in experiment 8 and also in previous investigations (Olesiuk and Van Roekel, 1960). It is significant that transmission of the infection through cohabitation of infected and susceptible chickens does not always occur even after prolonged periods of contact. Furthermore, a limited number of trials were unsuccessful in demonstrating transmission through indirect contact. The suspicious evidence of transmission obtained through force-feeding of feces from serologically positive birds may have some significance and this avenue should be explored further.

The authors feel that in some of these transmission trials in which no spread was observed that they may have been terminated too soon. Furthermore, since MG can be readily recovered from the upper respiratory tract of serologically positive birds attempts should be made to culture susceptible contact birds in a similar manner at the start and termination of a transmission trial, to ascertain if the organism can exist in the susceptible host without producing manifestations of the disease. If *Mycoplasma* are recoverable from such susceptible birds, their serotype should be ascertained.

In appraising these limited observations it would seem urgent to ascertain the var-
ious modes and rate of spread of *M. gallisepticum* and to further elucidate this aspect of the disease instead of continuing to express speculative opinions and posing them as facts in regard to disease dissemination.

The fact that MG-infection may be an egg-borne disease adds to the complexity and difficulty in controlling this malady. For eradication of the disease the logical approach is to break the cycle by establishing primary breeding and multiplier flocks free of the infection. Van Roekel *et al.* (1958) reported that this was feasible. In subsequent years the establishment and maintenance of MG-free flocks have also been reported by other workers (Chute and O'Meara, 1963; Chute and Stauffer, 1962; Chute *et al.*, 1964; Crawley and Fabey, 1955; Dunlop and Strout, 1956; Peterson, 1963, 1965). Various combinations of eradication procedures have been used. The serological methods employed in the testing of flocks have proved to be very effective when properly applied and when adequate sanitary and disease preventive measures are instituted. It seems unnecessary to enumerate in detail the precautionary measures that should be taken in an eradication program since they are similar to those recommended for other disease eradication programs (Anonymous, 1963, 1965; Brion, 1961; Chute and Stauffer, 1962; Peterson, 1963, 1965; Rosenwald and Adler, 1962). However, there are certain aspects of this problem that have a distinct influence on the eradication approach. The highly developed breeding lines and the size of the different breeding operations constitute a problem in eradication. However, a breakthrough has been made in eradicating the disease from a few large primary breeding operations (Peterson, 1963, 1964, 1965). In smaller breeding operations the eradication of the infection, as reported here, was not difficult nor too costly. The success in achieving the eradication goal is dependent on the determination of the flock owner, hatchery man, and disease control agent to develop and execute a sound, appropriate program for the breeding operation.

Small numbers of reacting birds that may be encountered in a flock should be investigated further by additional serological testing or other complementary tests. This is especially indicated for flocks that have originated from free sources. Also, it should be recognized that one is dealing with serological tests that have not been fully standardized for this disease, although they have been used very effectively thus far (Adler, 1954; Adler and Yamamoto, 1956; Aftosmis *et al*., 1960; Beckman *et al*., 1959; Chute *et al*., 1964; Cover *et al*., 1960; Dunlop and Strout, 1956; Hammar *et al*., 1958; Hofstad, 1957; Jacobs *et al*., 1954). When a *Mycoplasma* is recovered from doubtful reactors, such isolates should be investigated to determine their serotype, since several serotypes, many of them non-pathogens, have been reported (Adler, 1960; Edward and Kanarek, 1960; Kleckner, 1960; Yoder and Hofstad, 1964).

All birds on the premises, five months or older, should be tested since random testing may not be reliable, to detect traces of infection. It appears that if infection cannot be detected in birds tested at five months of age, that the flock may actually be free of MG-infection. However, in cases where the stock has originated from sources of questionable MG-status it would be prudent to not give such flocks the same rating for a disease-free status as negative stock that has come from sources with a validated disease-free status. Also, the premises on which the stock was reared should be taken into account in rating the flock.

The introduction of known infected or doubtful birds has been the most frequent cause for impeding the progress in eradica-
tion on a breeding farm or in a breeding corporation with many farms. Until this practice is avoided the eradication goal will not be attained. With the various approaches or combination of approaches that have been used to establish free flocks no real barrier for eradication should exist (Chute et al., 1964; Olson et al., 1962; Peterson, 1963, 1964; Stuart and Burns, 1963). Owners who have eradicated MG-infection from their breeding flocks claim that the performance of the negative stock is superior to that of the previous infected stock. Similar reports have been published by other workers (Chute and O'Meara, 1963; Chute and Stauffer, 1962; Moulthrop, 1962; Peterson, 1964, 1965).

The eradication results for *M. gallisepticum* presented in this paper reveal significant progress. Future progress will depend upon the industry's interest in eradication and the willingness and leadership of the research worker and control official to assist in developing and providing a sound program.

**SUMMARY**

Results of investigations concerning direct and indirect transmission of *M. gallisepticum* and the eradication of the disease in commercial breeding flocks are reported.

Contact transmission of the infection was demonstrated in four of eight experiments while no transmission was observed through indirect contact in three experiments. In the negative experiments *M. gallisepticum* was recovered from the upper respiratory tract of birds in the positive groups at the termination of some of the trials. The forced-feeding of feces collected from naturally infected mature bird induced suspicious serological evidence in susceptible chickens.

It was demonstrated that *M. gallisepticum* (S-6 type) infection is amenable to eradication from commercial breeding flocks on a practical basis. Flocks may be maintained free of this infection for as long as 11 consecutive years. In 1964, thirty-eight flocks were classified as serologically negative which represented 191,412 chickens or approximately one-third of the total breeding birds in Massachusetts.

Various aspects concerning transmission and eradication are discussed.

**REFERENCES**


O. M. Olesiuk, H. Van Roekel and D. H. Roberts

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Dierks, R. E., 1963. Personal communication.

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Effect of Carbon Dioxide Diluents on the Fertilizing Capacity of Turkey Spermatozoa

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ACK of information on a diluent containing carbon dioxide as a reversible inhibitor of metabolism of turkey semen induced Bajpai and Brown (1963a) to study the possibilities of using carbon dioxide diluents in storing turkey semen. Bajpai and Brown (1963a) reported that although the Illini Variable Temperature diluent (I.V.T.) and G-1 diluent (sodium glutamate substituted for sodium citrate in I.V.T.) containing carbon dioxide preserved turkey semen adequately in the laboratory both I.V.T. and G-1 diluent markedly inhibited the fertilizing capacity of turkey spermatozoa. However, in a subsequent study Bajpai and Brown (1963b) reported fairly good fertility (63 percent) with a glutamate diluent containing carbon dioxide and no egg yolk and poor fertility (35 percent) with a glutamate diluent containing 10 percent egg yolk by volume and no carbon dioxide. In the same year Harris et al. (1963) in a research note reported that the average fertility for turkey semen stored for six hours in a carbon dioxide diluent (a citric acid, sodium bicarbonate and sodium citrate diluent), computed on a weekly base, was 86, 72, 71, and 44 percent for weeks 1 to 4 after a single insemination. Bajpai (1965) reported 64 and 61 percent fertility for undiluted turkey semen and turkey semen diluted in a sodium glutamate sodium bicarbonate diluent. Both fowl and turkey semen contain large amounts of glutamic acid (Chubb and Cooper, 1962; and Ahluwalia, 1962) instead of