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The History of Avian Medicine in the United States. IX. Events in the History of Avian Mycoplasmosis 1905–70

John E. Lancaster[A] and Julius Fabricant[B]

[A]Toronto Road, R.R. #1, Port Hope, Ontario L1A 3V5 Canada
[B]Department of Avian and Aquatic Animal Medicine, NYS College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

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INTRODUCTION

In 1985, the President of the American Association of Avian Pathologists, Dr. C. W. Beard, drew attention to the fact that some writers of avian history are now deceased and that efforts should be made to continue their work. The main purpose is to record significant information and events that otherwise might be lost with the passage of time.

During 1985–86, the membership of the History of Avian Medicine Committee of the AAAP was reorganized, with the aim of developing historical accounts of two topics: Avian Mycoplasmosis and A History of the American Association of Avian Pathologists. The present report deals with the former topic and represents the work of a number of contributors, both members and nonmembers of the History Committee.

Readers of this historical account will be well aware of the very considerable amount of published material available on avian mycoplasmosis, including the proceedings of workshops, special meetings, and committees. As a result, it became very clear to the History Committee that reference could not be made to all individual writers. It was necessary, therefore, to make a selection among authors. In this task of selection, the Committee was very ably assisted by workers who for many years studied aspects of avian mycoplasmosis.

Nevertheless, it is recognized that the names of many experienced workers will not be found in this report, although their field of study may have been included. In addition, not all names mentioned in the text could be included in the list of references. Conversely, a few references are not cited in the text. The History Committee considered that the major objective was to record significant events and refer readers to the published bibliographies and reviews for additional references to the literature. These include a Bibliography and Index by Charles H. Domermuth and Judith G. Rittenhouse (29) and the Annotated Bibliography, November 1955, from Merck & Co., Inc. (63). Also, chapters in *Diseases of Poultry* (Iowa State University Press) and in *The Mycoplasmas* (Academic Press) provide historical information.

The history of avian mycoplasmosis may be divided into several stages that reflect our knowledge and interest in this series of diseases.

1. The recognition of infectious sinusitis of turkeys and chronic respiratory disease of chickens.
2. The identification of *Mycoplasma gallisepticum* (MG) as the primary etiologic agent of these clinical syndromes.
3. Recognition of the role of viruses and *Escherichia coli* as secondary infections leading to severe airsacculitis or so-called "complicated chronic respiratory disease."
4. The development of serological methods and isolation techniques for the accurate diagnosis of mycoplasma infection.

5. The recognition of the extent and distribution of mycoplasmosis in chickens and turkeys.

6. The recognition that egg transmission plays an important role in MG epidemiology.

7. The development of procedures and programs for treatment, control, and eradication of MG infection.

8. The recognition of infectious synovitis in chickens and turkeys, its etiologic agent *Mycoplasma synoviae,* and methods for handling this infection.

9. The recognition of *Mycoplasma meleagridis* in turkeys, its pathogenic potential, and methods for diagnosis and control.

1. THE RECOGNITION OF INFECTIOUS SINUSITIS OF TURKEYS AND CHRONIC RESPIRATORY DISEASE OF CHICKENS.

Two early accounts of the disease we now recognize as infectious sinusitis of turkeys were reported in England. The first was in 1905 by Dodd (28), who termed the condition "epizootic pneumoenteritis of the turkey." He noted that fowls running and feeding with the turkeys were not affected. Dodd reported the isolation of a fowl-cholera-like organism from the swellings on the head and from the lungs and heart. Hitchner (51), in reviewing the early literature, concluded that very likely Dodd was dealing with infectious sinusitis complicated with fowl cholera.

Two years later, also in England, G. S. Graham-Smith described "swollen head" in turkeys. He demonstrated transmission of the disease by direct contact and by inoculation of sinus exudate, which caused sinusitis. Bacteriological examination of sinus exudate yielded no specific organism. The disease resembled that described by Dodd (28) in that it appeared to be confined to turkeys and not transmissible to fowls by contact. However, the disease described by Graham-Smith differed in that mortality was insignificant. Yoder (101) considered the disease reported by Graham-Smith very likely to be MG sinusitis.

Yoder (101) considered Tyzzer (84) to be the first to describe sinusitis in turkeys in the United States, in 1926. Madsen (61) (1938) and Hinshaw (50) (1943) reported the occurrence and clinical pattern of turkey sinusitis in the western United States. In 1940, Hart (49) described its occurrence in Australia.

Infectious sinusitis of turkeys was studied in detail by Jerstad and Hamilton (52) in the state of Washington, where this disease had been a problem in two turkey breeding flocks during 1946. Jerstad and Hamilton considered the causal agent to be a virus and stated that:

"The virus has been found to occur naturally in turkey embryos from breeders infected with the respiratory form of the disease."

Little progress in the recognition of chronic respiratory disease of chickens was made until the other respiratory diseases of bacterial and viral origin were described. A major step was the isolation of the causal agent of one form of infectious coryza by De Blieck (23) (1931), of the University of Utrecht, The Netherlands. This organism was termed *Bacillus haemoglobinophilus coryzae gallinarum* by De Blieck, later to be classified as *Haemophilus paragallinarum.*

The terms coryza and infectious coryza were used by J. B. Nelson, who studied respiratory disease in domestic fowl and the laboratory rat and mouse at the Rockefeller Institute. Nelson published at least 10 papers on avian respiratory disease from 1933 to 1938. Most of these reports appeared in the Journal of Experimental Medicine (67,68). In addition, infectious coryza was studied by Beach and Schalm (10), who reported on the clinical manifestations and transmissibility. Apart from the work of Beach and Schalm, for many years little attempt was made to repeat Nelson's findings.

It is generally accepted that the paper by Nelson (66) entitled "Cocco-bacilliform bodies associated with an infectious fowl coryza" represents the first publication on chronic respiratory disease as a diagnosable disease.

An undated and probably unpublished general review was prepared by Nelson entitled "Bacteria of the Pleuropneumonia Group," in which he described his own work in these words:

"In the early 1930's I began to work with an infectious coryza of chickens. Films of exudate showed grouping of minute Gram-negative elements which were spherical or slightly elongated. They were filterable through large pored collodion membranes but were not cultivable in such nutrient media as I then used. They grew very well, however, in a tissue culture composed of minced chick embryo tissue suspended in Tyrode's solution. None of my associates at Rockefeller were able to identify these organisms and I called them descriptively coccobacilliform bodies. The report of this work was published in 1935 some months after Klieneberger's paper which introduced the term PPLO (pleuropneumonia-like organism)."

Dr. E. Klieneberger worked on *Streptobacillus moniliformis* at the Lister Institute in London, England, and noted the presence of small morphologic elements that could be separated from the parent growth and produced minute colonies on serum-agar. For these L forms, Klieneberger suggested the term pleuropneumonia-like organism, or PPLO. Eventually it was recognized that the coccobacilliform bodies described by Nelson were in fact pleuropneumonia-like organisms.

Before his death, Dr. Melvin S. Hofstad, of Ames, Iowa, prepared the following summary of the work of Nelson for the present historical review. According to Hofstad:

"There is little doubt that the first observation of *Mycoplasma gallisepticum* infection in chickens was made in 1933 by Nelson. He found that there existed 3 types of coryza with the following features: 1. a rapid onset and short course; 2. a slow onset and prolonged course; 3. a rapid onset and prolonged course. As we know today, the first was due to *Haemophilus paragallinarum* (*H. gallinarum*), the second due to *M. gallisepticum* and the third due to a mixture of the two agents. In a later publication, Nelson (67) (1936) was able to demonstrate coccobacilliform bodies in the nasal exudate from infected birds. He later was able to grow the coccobacilliform bodies in 3-to-4-day-old embryonating chicken eggs and also in tissue cultures and proved that the cultivated organisms were infectious for chickens causing the disease of slow onset and prolonged course.

"Nelson (68) (1939) demonstrated growth of the coccobacilliform bodies in supernatant fluid from sedimented embryo tissue, in media heated to 100 degrees Celsius for 1 hour to kill tissue cells, and in media in which the cells had been inactivated by storage for 4 weeks. On the basis of their cultural features, Nelson suggested a bacterial nature for the coccobacilliform bodies but did not believe them to belong to the pleuropneumonia group of organisms because the coryza bodies failed to exhibit the complex morphology which characterizes that group. However, Smith *et al.* (81) in their electron micrograph studies of pleuropneumonia-like organisms, obtained Nelson's coccobacilliform bodies and found them typical of the pleuropneumonia-like organisms of human derivation."

The term "chronic respiratory disease" was first used by Delaplane and Stuart (27) in the title of their paper published in 1943. That paper described a disease in chickens characterized by nasal discharge, respiratory rales, slow rate of spread, and persistence of symptoms. The causal agent was propagated in fertile chicken eggs through 35 passages and reproduced typical symptoms in mature birds. These authors considered the agent to be a virus distinct from laryngotracheitis and infectious bronchitis viruses. In retrospect, the disease studied by these authors was most likely MG infection.

During 1945, an outbreak of a chronic respiratory disease in laying hens in New York State was studied by Hofstad (unpublished data). In the following year he observed additional cases at the University of Connecticut and was able to transmit the disease to experimental chickens. In 1947, the disease was first recorded in Iowa.

Chu (15) (1954), working in England, reported the occurrence of respiratory diseases in poultry associated with coccobacilliform bodies as described by Nelson, in which the air sacs were involved. He concluded that the infectious coryza studied by Nelson and the chronic respiratory disease of Delaplane and Stuart were likely the same disease. The difficulties faced by workers during the 1940s was emphasized by Delaplane (26), who stated:

"The symptoms of the chronic respiratory disease as seen in mature chickens cannot be distinguished from those resulting from infectious bronchitis or Newcastle disease. However, the chronic respiratory disease differs in that it is characterized by a slow rate of spread."

Delaplane and Stuart (27) (1943), in their original description, mentioned rhinitis, tracheitis, and loss of weight. In contrast, Grumbles *et al.* (45) found that in chickens the disease could be almost inapparent. Chu (16), who studied 65 outbreaks of chronic respiratory disease, found that chicks 3 weeks old as well as laying hens were affected, the incubation period following natural exposure was usually 10 days to 2 weeks, and the infraorbital sinuses were usually affected.

Van Roekel *et al.* (90) (1957) reported the results of 69 field cases submitted during 1952–53 for serology, virus isolation, microbiological procedures, and histological examinations. The most common gross lesion observed was exudation in the lumen of the trachea, usually with thickening of the tracheal mucosa.

2. THE IDENTIFICATION OF *MYCOPLASMA GALLISEPTICUM* (MG) AS THE PRIMARY ETIOLOGIC AGENT OF THESE CLINICAL SYNDROMES.

As previously mentioned, early etiologic studies started from several different directions. Nelson (67) described a chronic coryza-like disease caused by coccobacilliform bodies. Delaplane and Stuart (27) described a chronic respiratory disease associated with tracheitis and airsacculitis due to an egg-passaged agent, presumed to be a virus. In 1948, Delaplane (25) described the lesions produced in chicken embryos. Van Roekel *et al.* (89) described the isolation of the "chronic respiratory disease agent" in chicken embryos. Fabricant (33) (1951) also described a similar agent isolated in chicken embryo yolk sacs from layer replacement chickens, which continued to show a chronic tracheitis and airsacculitis following a "controlled exposure" program for infectious bronchitis immunization. This agent was at first suspected to be an ornithosis-like agent.

The common thread binding these diseases was found when Delaplane (25) (1948) showed that the agent causing chicken chronic respiratory disease was capable of inducing sinusitis in inoculated turkeys.

In 1952, Markham and Wong (62) made the landmark discovery that chronic respiratory disease of chickens and infectious sinusitis of turkeys were caused by a pleuropneumonia-like organism (PPLO) that could be cultivated in a cell-free culture medium. This was soon confirmed by Van Roekel and Olesiuk (88).

Van Roekel and Olesiuk published extensively on the etiology of chronic respiratory disease in chickens, including studies on transmission, host susceptibility, route of infection, response to antibiotics, and immunity. These publications described the work conducted at the University of Massachusetts during the period commencing in 1950 [Van Roekel *et al.* (89) 1952] and continuing for approximately 20 years.

A second landmark discovery was the differentiation by Adler and Yamamoto (2) (1957) of pathogenic and nonpathogenic PPLO. They isolated two distinct strains of PPLO from a turkey with infectious sinusitis. One strain produced sinusitis in turkeys and respiratory lesions in chickens; the other apparently was not pathogenic. In collaboration with Berg and Fabricant, other studies on this general topic were published (4,5).

At this point, it was realized that the problem of diagnosis was complicated by the frequent occurrence of pathogenic PPLO (MG) as part of a flora often mixed with nonpathogenic species of PPLO. This work was extended during 1958, when Yamamoto and Adler (95,96) reported their studies on antigenic analysis, comparative pathogenicity, and cultural, biochemical, and morphological characteristics of avian PPLO. For this work, a total of eight strains (isolates) were used. Among these isolates was the S6 strain, originally isolated by D. V. Zander in 1954 from the brain of a turkey showing nervous signs. Zander (105) recorded that re-inoculation of the S6 strain into turkeys caused nervous symptoms and locomotor disturbances. Yamamoto and Adler showed that the strains of PPLO of avian origin available to them could be separated into five groups according to morphological, physiological, and antigenic characteristics.

The term pleuropneumonia-like organism predominated in the published literature during most of this period. Not until 1960 did authors begin to refer to the organism as mycoplasma. This change resulted from the paper by Edward and Kanarek (32) (1960), who proposed the name *Mycoplasma gallisepticum* for the PPLO that caused chronic respiratory disease in chickens and infectious sinusitis in turkeys.

The pleuropneumonia group of organisms was first included in the Bergey Manual of Determinative Bacteriology in 1948. It was unfortunate that the avian mycoplasmas were not described in the Bergey Manual until the 7th edition (1957). The type strain described at that time was *Mycoplasma gallinarum* as reported by Edward and Freundt (31) (1956). That organism was a nonpathogenic isolate recovered from the upper respiratory tract of a fowl and later classified as a B serotype of Kleckner (57) (1960) or the K18B group of Fabricant (34). The typical pathogenic species of the avian mycoplasmas was named *Mycoplasma gallisepticum* by Edward and Kanarek (32) and was listed in the Bergey Manual 8th edition (1974). That isolate is the avian serotype S6 of Adler *et al.* (5) and Fabricant (34) (1960).

The diversity of avian PPLO was demonstrated by Fabricant (34), who used hyperimmune antisera to classify 170 cultures. These isolates fell into six different groups plus a group of unclassified cultures. Eighty-one—nearly half of the isolates—were identified as the S6 pathogenic type. Kleckner (57) (1960) characterized a total of eight serotypes. Yoder and Hofstad (103) described the Iowa 695 serotype (now designated *M. iowae*). Roberts (79) (1964) described two more serotypes from poultry and named *M. anatis* from ducks. Grumbles *et al.* (46) (1964) characterized several of the avian serotypes using tissue-culture systems. Nineteen serotypes ("A" through "S") have now been combined and reduced to some 12 serotypes, all of which have received genus and species designations.

The ultrastructure of MG was explored by different research groups. However, the report by Domermuth *et al.* (30) presented a logical description of all components of the organism. Ribosomes were clearly evident within some sections.

A controversial series of reports concerned the apparent reversion of some avian mycoplasma cultures to vegetative bacteria. This suggested that some of the PPLO (later named mycoplasma) were actually L-forms of bacteria. However, it now appears that the majority of the bacteria from so-called reverted cultures were gram-positive bacteria which were present in some broth cultures of true mycoplasma, but were almost inhibited from growth by the presence of high levels of thallium acetate

and penicillin. The death knell of this concept was not rung until many years later, when DNA homology techniques proved there was no relationship between these PPLO and their suspected revertant forms.

The gross pathology and histopathology of mycoplasma infections of poultry were studied by Jungherr *et al.* (55) (1953), Olesiuk and Van Roekel (69) (1960), Rhoades *et al.* (78) (1965), and Barber (9) (1962). Hematological studies were reported by Fedde and Pomeroy (41), and Chute (17) (1960) described the pathology of mycoplasma infections in chicken embryos.

3. RECOGNITION OF THE ROLE OF VIRUSES AND *ESCHERICHIA COLI* AS SECONDARY INFECTIONS LEADING TO SEVERE AIRSACCULITIS OR SO-CALLED "COMPLICATED CHRONIC RESPIRATORY DISEASE."

As indicated in the previous section, Delaplane and Stuart (27) (1943) introduced the term "chronic respiratory disease." However, Jungherr and Luginbuhl (54) noted that the term "air-sac infection" had come into common use about 1947. At that time, the evisceration of commercial broilers often showed the presence of widespread inflammatory exudate over the internal organs and within the air sacs. Jungherr and Luginbuhl (54) (1952) recognized that the condition termed air-sac infection was "etiologically related to chronic respiratory disease of chickens, and to sinus and air sac infection of turkeys." Nevertheless, in retrospect, the exact etiology was still uncertain, because in 1952, the authors stated that: "The essential air-sac infection agents are twofold in nature according to current thought, namely a pleuropneumonia and an ornithosis-like agent, but their relative importance and their relationship to other non specific and specific factors is not known."

The possibility of "air-sac infection" or "chronic respiratory disease" being associated with Newcastle disease was of concern to the U.S. Department of Agriculture. As a result, on March 11 and 12, 1952, the Pathological Division of the then Bureau of Animal Industry of the U.S. Department of Agriculture initiated a "Conference to Develop a Coordinated Research Program on Air Sac Infection of Broilers" in Washington, D.C. The conference chairman was B. T. Simms, Chief, Bureau of Animal Industry. This conference was attended by members of the poultry industry and federal and state governments. There were 34 participants from 20 states. In his introduction, Dr. Simms reported that heavy losses had occurred in broiler houses in the Delmarva area. H. W. Schoening, of the USDA, noted that the conference was similar to the one held in Baltimore in 1946 to develop a coordinated research program on Newcastle disease. The three major topics were:

1) "Air-sac infection," definition and criteria for diagnosis.
2) Consideration of a national research program.
3) Research and control program to be developed.

During October 1952, another meeting was held in Washington, D.C., in order to "Review the Air Sac Disease Program." A copy of the report of this meeting has not been located, but unpublished notes prepared by the late H. E. Adler are available. These notes indicate that the funds required to undertake the research program established at the March 1952 meeting were $160,000 for the fiscal year 1952. Difficulty was experienced in the allotment of the funds by the designated date of June 20, 1952. Adler also recorded that:

"Another $75,000 was made available for the fiscal year 1953 by congressional appropriation."

The meeting of October 1952 dealt at length with the diagnosis of air-sac disease, the transmission, and antibiotic treatment. Reports were presented from approximately 20 states. In addition, the results of field studies were described by C. H. Thompson, of the Bureau of Animal Industry. A committee composed of E. L. Jungherr, H. Van Roekel, W. A. Boney, and H. E. Adler was established to exchange representative cultures and to study their characteristics.

For many years, the poultry industry and pathologists associated with it continued to use the term "air-sac infection." No doubt this was due to the fact that during this period, most outbreaks of respiratory disease were diagnosed on the basis of symptoms and lesions. As a result, the early material available on economic loss is based largely on clinical and gross pathological findings.

At the USDA Conference on *Air Sac Infection of Broilers* held in 1952, a number of reports were presented on economic loss in different states. I. M. Moulthrop, from Maryland, noted that,

"This condition reached its peak in the winter of 1950–51," and probably "between 25 and 30 percent of birds died from air sac infection during a three month period of 1951–52."

The report from Maryland also indicated the presence of Newcastle disease, infectious bronchitis, and infectious laryngotracheitis viruses. E. Jungherr, from Connecticut, described his first contact with the disease in 1950. This was in a laying flock of New Hampshire birds that failed to show respiratory symptoms but showed insufficient egg production. In 1951, repeated outbreaks occurred in broiler flocks in Connecticut. Mortality varied from very low up to 30%. E. F. Waller, from Delaware, reported that affected flocks showed an overall mortality of 25 to 30%. During the winter of 1951–52, chronic respiratory disease became enzootic in the Delmarva area. In addition to the mortality, there was a marked decrease in feed consumption and carcass quality. These factors cost the Delmarva poultryman about $20 million over an 8-month period. T. B. Clower, from Georgia, estimated mortality in affected flocks at approximately 35%. A similar level of mortality was reported by H. E. Moses of Indiana. H. L. Chute (Maine), H. Van Roekel (Massachusetts), B. F. Cox (N. Carolina), and M. L. Miner (Utah) reported either poor feed conversion or low feed consumption.

The national picture was summarized by J. F. Sullivan, C. H. Thompson, and O. L. Osteen (1956) as follows:

"The average annual mortality from chronic respiratory disease in chickens from 1942 through 1951 was more than 14 million birds. Each year, additional losses are due to the decreased gains and lowered feed utilization that accompany the disease, extra time needed to bring broilers to market, a larger number of unmarketable birds and lower egg production and hatchability."

Speakers at the Washington conference in 1952 concluded that "air-sac infection" of broilers first became of significance in January and February 1947 on the Eastern shore of the United States. Some pathologists considered the disease to be associated with extremes of heat or cold; others reported a high incidence during fall and winter. It was also very clear from the conference discussion that other respiratory viruses, especially Newcastle disease and infectious bronchitis viruses, had an effect on the "clinical syndrome and pathologic changes in the respiratory tract."

The 1952 conference on air-sac infection established seven committees to report back to the conference. One committee, under Van Roekel as Chairman, examined the problem of etiology. A number of studies were suggested. Recommendation number V1 was as follows:

"Influence of seasonal, management, and other factors on chronic respiratory disease. Data should be collected through laboratory experiments and field observations regarding the influence of seasonal, management, and other factors on the incidence, severity, and course of the disease."

The South Central Poultry Research Laboratory of the USDA at Mississippi State was established in 1964 to examine the problem of chronic respiratory disease from the aspects of management and environment. Reports available at that time showed that chicken broiler carcass condemnation was higher during the winter.

Experimental production of mycoplasma infections in poultry was reported by a number of research groups. The complexity of the etiologic agents and the sequence

of infection evolved from a series of studies involving various bacteria and viruses. Gross (43,44) defined the importance of *E. coli* experimentally, especially in sequence with Newcastle disease or infectious bronchitis viruses.

A definitive study by Fabricant and Levine (36) (1962) compared the roles of MG, infectious bronchitis virus, and *E. coli* alone and in various combinations and demonstrated the importance of a primary MG infection for the subsequent development of *E. coli* airsacculitis. These authors also demonstrated that airsacculitis spread only by contact of susceptible chickens with chickens previously infected with MG. This indicated that it should be possible to control the field airsacculitis problem by programs designed to eliminate MG.

4. THE DEVELOPMENT OF SEROLOGICAL METHODS AND ISOLATION TECHNIQUES FOR THE ACCURATE DIAGNOSIS OF MYCOPLASMA INFECTION.

In 1945, Van Herick and Eaton (87) demonstrated that a PPLO isolated from chicken embryos was capable of causing hemagglutination *in vitro,* and that the blood serum of the corresponding dams contained hemagglutination-inhibition (HI) antibodies. Following that report on the presence of circulating antibodies, there appeared to be an interval of 6 years or so before Jungherr and Luginbuhl (54) (1952) proposed the possible eradication of PPLO in poultry based on a testing program similar to that for pullorum disease.

The following year (1953), Adler (1) published a paper entitled, "Preliminary report on a slide agglutination test for the pleuropneumonia-like agents associated with infectious sinusitis of turkeys and chronic respiratory disease of chickens." Adler concluded that positive serum might be used to identify suspected pleuropneumonia-like organisms isolated from field cases.

These early studies by Jungherr and co-workers at Storrs, Connecticut, and by Adler when at Washington State College stimulated appreciable interest, and within one year, no fewer than six different reports on serological tests were published. The authors involved included H. E. Adler, J. F. Crawley, J. F. Fahey, E. M. Gianforte, R. E. Jacobs, and F. H. White.

As a result of the serological testing of a number of chicken and turkey flocks, Jungherr *et al.* (56) (1955) concluded that serological tests were a sensitive and practical tool for recognizing avian PPLO infections.

Many studies were conducted on the growth requirements of the organism. These studies were needed to facilitate the isolation of the organism, the identification of the disease, and the growth of the organism in quantity. This in turn permitted better examination of the characteristics of the organism and provided the quantities of antigen needed to develop test procedures for diagnosis and control measures. The fastidious growth requirements of mycoplasma were recognized early, and appreciable research during this period was devoted to variations in liquid and solid media to promote better growth.

Studies continued on perfecting serological tests for the detection of infected carriers of mycoplasma. Various antigen preparations were developed for the diagnosis of MG infection by serological testing. Crawley (21) described the use of the HI test, which he had previously developed. Antigen production and rapid serum plate or tube agglutination testing was reported by a number of workers during the period 1962–65. Corstvet and Sadler (19) (1964) described the use of a fluorescent-antibody procedure to identify MG organisms. Boyer *et al.* (12) (1960) apparently were the first to describe nonspecific reactions to the rapid serum plate agglutination test. This problem was to continue during the succeeding years. Olson *et al.* (74) (1965) studied the antigenic relationship of *M. synoviae* and MG and showed that cross reactions

to MG plate antigen were often noted when serum was tested from chickens during the first 2 or 3 weeks of *M. synoviae* infection.

Serological tests were being developed and improved to aid in the detection of outbreaks in the field. Vardaman and Yoder (92) and Rhoades (77) were among the workers who contributed to this development.

Frey *et al.* (42) developed a broth medium that enabled laboratories to culture the organism for diagnostic confirmation. Frey's medium remains the most popular mycoplasma culture medium in use today.

5. THE RECOGNITION OF THE EXTENT AND DISTRIBUTION OF MYCOPLASMOSIS IN CHICKENS AND TURKEYS.

By 1950, Delaplane (26) concluded that in the United States, chronic respiratory disease of chickens and turkeys could "be considered as having a national rather than regional distribution." Cover and Waller (20) reported in 1954 that evidence of mycoplasma infection was found in 75% of breeding flocks tested.

During the early 1960s, reports were published on the occurrence of mycoplasma infections in many countries throughout the world. By 1970, the host range of mycoplasma infection was reported to include chukar partridges, parakeets, and pigeons.

By the time sufficiently accurate diagnostic methods were available to determine the extent of MG infection, a significant difference between the chicken and turkey industries had occurred. Chicken breeding had changed from pure line breeding in relatively small flocks to double hybrid breeding programs on very large specialized farms. In the course of making this change, chickens were introduced onto the breeding farms from many sources, and MG infection became prevalent in practically all of the commercially useful breeding chickens. On the other hand, turkeys were still on pure-line breeding programs. Consequently, it was possible to find many turkey breeding flocks that were free of infection.

6. THE RECOGNITION THAT EGG TRANSMISSION PLAYS AN IMPORTANT ROLE IN MG EPIDEMIOLOGY.

One of the earliest studies on egg transmission involved infectious sinusitis of turkeys due to MG (52). In a subsequent report, Jerstad *et al.* (53) inoculated sinus exudate into the allantoic sac of twenty-eight 14-day-old turkey embryos. Eight of twelve hatched poults survived, and five of these showed typical sinus swelling when 6 to 9 weeks of age. From this, it was concluded that the causal organism had been transmitted via the egg.

Transmission of the chronic-respiratory-disease agent through the chicken egg was examined by Van Roekel *et al.* (91) during 1952–53. Those workers concluded that, "The chronic respiratory disease agent had been detected in embryos and chicks which originated from infected breeding stock."

Also during 1952–53, at the Delaware Agricultural Experiment Station, Newark, a number of samples of pipped live or dead chicken embryos were examined from four commercial hatcheries by Cover and Waller (20). The causative agent of chronic respiratory disease was found in 75% of 70 egg lots tested.

The following year, Fahey and Crawley (39) reported similar findings:

"The PPLO were isolated from infected hens, from unpipped eggs dying immediately prior to hatching and from cull chicks obtained immediately after hatching."

In view of these findings, Fahey and Crawley concluded "that control of pleuropneumonia-like infections in poultry be started in the breeder flock."

Fabricant and co-workers (37,38) conducted an extensive series of studies on the patterns and mode of MG egg transmission. They observed that MG could be egg-transmitted in individual trap-nested chickens for periods over 6 months. They also determined that this was not due to transovarian infection but was associated with

persistent mycoplasma infection in the infundibulum and upper end of the oviduct and resultant contamination of the yolk-sac membrane.

7. THE DEVELOPMENT OF PROCEDURES AND PROGRAMS FOR TREATMENT, CONTROL, AND ERADICATION OF MG INFECTION.

When it was found that MG was susceptible to several antibiotics in laboratory tests, numerous trials were made to test the efficacy of antibiotic treatment of flock infection by injection, or by administering the antibiotic in feed and water. The results were varied, depending upon the complicating infections present in the flock and the resistance of the organism to the antibiotic. Such procedures were also expensive and often were not economically justified by the results obtained. It soon became obvious that successful control of MG depended on the production of MG-free day-old chicks and poults. This could only be achieved by the production of MG-free breeding stock. Control of mycoplasmosis by testing and elimination of individual carriers, as had been done in eradicating pullorum-typhoid, was not feasible. Testing was useful in detecting flock infection, and this aided control of the disease by avoiding positive flocks in the breeding program.

As previously indicated, because of differences in the type of breeding program, the extent of MG infection differed in turkeys and chickens.

In the turkey industry, there were numerous isolated breeding flocks, many of which were not infected with MG. By a combination of careful clinical evaluation and serological testing, all infected breeding flocks were eliminated from turkey breeding programs, and the disease was eradicated with relatively little difficulty. The report by Hall *et al.* (47) in 1961 concerned the eradication of MG infection in turkeys by hatching only from serologically negative breeders; this has been the key to continued progress toward eradication of MG in turkeys.

Chicken production, on the other hand, depended on a relatively few very large breeding farms, which were all infected with MG. This meant that programs had to be devised to produce MG-free chicks from infected dams or in other words to break the cycle at the stage of egg transmission. Crawley and Fahey (22) (1955) described "A Proposed Plan for the Control of Chronic Respiratory Disease of Chickens." This plan involved the injection of antibiotics into infected hens, the collection of eggs soon afterward for replacement stock, and the rearing of that stock on clean and isolated premises. The PPLO HI test was an integral part of this proposed plan.

Antibiotics were widely studied to eliminate the development of the infection in young chicks. Adler *et al.* (3) (1956) reported efforts to control egg-transmitted PPLO by antibiotic medication of the foundation stock. Similar attempts were made by other workers. Finally, Peterson (75) (1965) reported the apparent eradication of MG infection from breeder chickens by the repeated injection of tylosin in oil simultaneously with chlortetracycline in feed potentiated with terephthalic acid.

It was eventually realized that antibiotic treatment of chicks or breeders significantly lowered the rate of infection and egg transmission but was not a uniformly successful method of eliminating MG infection.

Two other approaches in the control of egg transmission were examined: immunization and antibiotic treatment of hatching eggs rather than birds.

Adler *et al.* (6) (1960) demonstrated that chickens and turkeys which had recovered from mycoplasma infection were reasonably resistant to re-infection. However, vaccines prepared from sonicated or formalinized cells were not very effective in protecting turkeys from infection. A series of reports were published on the use of live, virulent MG organisms as live vaccines to infect young replacement chickens, which then might be less likely to be actively infected as adults. Fabricant and Levine (37) (1963) studied a live vaccine as a method to prevent egg transmission from adults to their progeny. This procedure proved effective under laboratory conditions but

was not considered practical under field conditions because of the difficulty of producing sufficiently virulent vaccine and the danger of acute secondary *E. coli* infection.

Chalquest and Fabricant (13) explored the possibility of controlling PPLO by eliminating the egg-transmitted infection by dipping eggs in antibiotic solutions. Trials were made by warming the eggs and then dipping them in cold solutions containing erythromycin or streptomycin. Erythromycin proved to be the most effective in reducing the number of isolations from treated eggs. Levine and Fabricant (60) (1962) studied erythromycin solutions and tylosin solutions; Hall *et al.* (48) (1963) studied spiramycin and erythromycin solutions; Olson *et al.* (71) (1962) used erythromycin. The use of cold solutions of tylosin was reported by Voeten (93) and Yoder and Hofstad (104). Most of the reports noted very favorable elimination of egg transmission of MG if adequate levels of the antibiotics were employed in very cold solutions for 15 to 30 minutes, or by the pressure differential (vacuum) method of Alls *et al.* (7). The development by Yoder (102) of heat-treating fertile eggs to decrease the organism was a big step towards the goal of eradication, and Smit and Hoekstra (80) reported on a procedure for injecting hatching eggs with tylosin.

During the early-to-mid 1960s, a series of meetings were conducted on a national scale to address the problems of diagnosis, control, and possible eradication of mycoplasma infections in poultry. The Animal Health Division of the Agricultural Research Service of the USDA sponsored two very important meetings during that time. Their reports and a related report sponsored by the Animal Disease Eradication Division did a great deal to bring together people to review our knowledge at that time and propose continued research needs, in addition to making specific recommendations for the diagnosis and control of MG in chickens and turkeys. The report of the Second Study Group in 1965 (85) still remains valid in 1988. This report was prepared by the Mycoplasmosis Committee of the American Association of Avian Pathologists, in cooperation with federal and industry representatives. A slightly modified version of this document was published by the USDA-ARS as "Recommended guidelines for the control and eradication of *Mycoplasma gallisepticum* infection in chickens and turkeys" (86). These guidelines were eventually incorporated into the National Poultry and Turkey Improvement Plan. Later, similar programs for the control of *M. synoviae* and *M. meleagridis* were added to the program. These combined efforts have been very extensive and extremely successful in eradicating MG from primary poultry breeding and reproducing flocks.

8. THE RECOGNITION OF INFECTIOUS SYNOVITIS IN CHICKENS AND TURKEYS, ITS ETIOLOGIC AGENT *MYCOPLASMA SYNOVIAE,* AND METHODS FOR HANDLING THIS INFECTION.

Infectious synovitis was first recognized as a distinct disease in broilers in 1954 by Olson *et al.* (70). Snoeyenbos and Olesiuk (83) described a similar disease in turkeys. Lecce *et al.* (59) found the agent to be a PPLO. Chalquest and Fabricant (14) determined that the synovitis agent, *M. synoviae,* was unlike MG in that it required an additional growth factor of diphosphopyridine nucleotide.

These authors also described special media and techniques for the isolation and cultivation of the mycoplasma that Olson *et al.* (73) (1964) named *M. synoviae.* Olson *et al.* (72) (1963) described the preparation of plate agglutination antigens.

In the period 1957 to 1965, Olson and his colleagues presented a series of eight papers on the use of antibiotic medication to treat and alleviate infectious synovitis outbreaks under field conditions.

Soon after the identification of infectious synovitis, in 1954, there was some evidence suggesting that the causal agent could be transmitted through the egg (94). Initially, considerable difficulty was encountered in experimentally infecting mature chickens with the agent of infectious synovitis (82). However, the results provided

evidence that infectious synovitis may be transmitted by egg. Eleven years later, Chute *et al.* (18) reported field studies involving more than 700,000 chicken broilers and concluded that embryo transmission of *M. synoviae* was probable.

Yoder (102) (1970) described a heat-treatment procedure for the elimination of *M. synoviae* from hatching eggs. Vardaman and Yoder (92) (1970) developed a *M. synoviae* hemagglutinating antigen for use in the HI test. Soon afterwards, procedures for the control of *M. synoviae* were added to the National Poultry and Turkey Improvement Program.

9. THE RECOGNITION OF *MYCOPLASMA MELEAGRIDIS* IN TURKEYS, ITS PATHOGENIC POTENTIAL, AND METHODS FOR DIAGNOSIS AND CONTROL.

Adler *et al.* (5) (1958) first recognized that a new variety of PPLO designated "N" type was associated with airsacculitis in day-old poults. These organisms were later classified as *M. meleagridis* by Yamamoto *et al.* (98).

In 1964, Bigland *et al.* (11) reported results based on the presence of gross lesions of airsacculitis in cull day-old turkey poults and concluded that egg transmission of "N" strain mycoplasma (*M. meleagridis*) increased with the length of lay. Two years later, Mohamed *et al.* (65) isolated *M. meleagridis* from the uterus and vagina of turkey hens. Although no isolation was made from the ovary or infundibulum, those authors concluded that:

"In the turkey hen, Mycoplasma have the tendency to localize in the reproductive tract, specifically in the uterus and vagina, and in this location could be the source of egg transmission."

This conclusion was extended by Yamamoto *et al.* (99) (1965), who showed that "N" strain mycoplasma (*M. meleagridis*) could be isolated from the reproductive tract of virgin turkeys. Reports by Kumar *et al.* (58) (1963) and Bigland *et al.* (11) (1964) confirmed the presence of these mycoplasmas in airsacculitis of young turkeys in the absence of MG. Yamamoto and Bigland (97) (1964) reported the establishment of a small flock of turkeys free from "N" strain mycoplasma. Those authors made use of uninfected turkeys to prove the pathogenicity of the "N" strain organisms in producing airsacculitis in young turkeys.

The disease was determined to be sexually transmitted (100) but was not related to infertility. Skeletal deformities in older birds were related to early infections with *M. meleagridis* (76).

In 1967, the tube agglutination test was shown to be valuable for the detection of infected turkeys (64).

CONCLUSION

It is relevant to this history on avian mycoplasmosis to recall a comment made by the late Dr. J. Frank Witter in the first of this series on the History of Avian Medicine, published in *Avian Diseases* in 1976 (Vol. 20:621–630). Dr. Witter stated:

"History is a compass bearing that gives a sense of direction for a continuing journey over winding roads. A look at the past is not a backward turn; it can lay the foundation of future progress."

Respiratory diseases of poultry were described as serious problems in publications available during the last two decades of the 19th century. At that time and for many decades of the present century, there was much confusion concerning the etiology of the different respiratory diseases of poultry.

The history of avian mycoplasmosis serves as an example of the unravelling of a complex clinical disease. The present history describes the studies conducted on the etiology and the division of the clinical disease into distinct entities. The mortality, lack of growth, and condemnations on processing placed considerable demands on

most segments of the poultry industry. Thus, this history records the studies made in the laboratory and the application of these findings in the field. These efforts resulted in the recognition of different species of avian mycoplasma. The means of spread of these mycoplasmas was an early finding, and this in turn led to extensive control and eradication measures.

As the laboratory and field work progressed, there were also important changes occurring within the poultry industry. Fewer and larger chicken and turkey farms were being established. These changes were associated with more centralized decision making with respect to disease-control measures.

Finally, the control measures against avian mycoplasmosis that were adopted during a relatively short period of time were influenced by the experience gained in the control and eradication of pullorum disease. Thus, to paraphrase the quotation from Witter (1976) given above: a look back at pullorum disease laid the foundation for progress in the control and eradication of avian mycoplasmosis.

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