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Clinical, Cultural, and Serologic Observations of Avian Mycoplasmosis in Two Chicken Breeder Flocks[A]

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Summary

Two chicken breeding flocks, from different breeding lines, were studied throughout their growing and laying period. Detailed observations were made of M. gallisepticum (MG) and M. synoviae (MS) plate test reactions and hemagglutination-inhibition (HI) test results. Both the breeders and their progeny were cultured for mycoplasma. Neither flock had an MG plate reactor rate much in excess of 60 percent of the birds tested. Also, the speed and degree of plate agglutination was not as intense as that commonly associated with characteristic outbreaks of MG. The MG-HI titers were rarely 80 and often less than 40 or 20. MG was, however, successfully isolated from both breeders and progeny originating from these two flocks. Observations of these flocks disclosed that: (1) Negative plate tests of large numbers of day-old progeny may sometimes be found in flocks known to be infected with MG; (2) It may be very difficult to consistently isolate MG from some infected flocks; (3) Overgrowth of M. gallinarum may interfere with successful cultivation of MG and; (4) A persistent breeder flock reactor rate of 10 percent or greater for a 4-12 week period is a strong indication of MG infection despite weak or negative HI test results.

Introduction

During the past five years of M. gallisepticum (MG) testing, flocks were occasionally encountered that did not reveal the expected reaction pattern.

Some flocks showing plate reactions did not develop the usual 80-100 percent reactivity rate within a short period of time with many reactors developing positive hemagglutination-inhibition (HI) titers.

It became increasingly difficult to prove that these were unusual forms of MG infection or were simply non-specific reactions. Additionally, results were not always in agreement with some private or commercial laboratories. In recent years the scientific community has been divided on the significance of these unusual testing reactions.

Flock test reports were closely followed and some revealed disturbing reaction patterns. During 1974 and 1975 we intensified our investigation of two particularly interesting flocks originating from different breeding lines. Reactor breeders and their progeny were submitted to laboratories in and out-of-state for cultural examinations.

In Pennsylvania approximately 60,000 chicken sera originating from over 160 different breeding flocks are tested annually for MG. A valid interpretation of test results is critical to the survival of these flocks.

Report

Summaries of laboratory reports for the two flocks are presented in tables 1 and 2. M. synoviae (MS) reactions in both flocks A and B have been essentially negative throughout the entire testing period. The reported isolations of MG were confirmed by biochemical and/or FA examinations by one or more recognized authorities. In several instances infection was additionally confirmed by bird inoculation and subsequent serology.

MG plate reactions persisted throughout the entire growing and laying period of Flock A (Table 1) but never approached the level of 80-100 percent. The intensity of agglutination and the speed at which it developed was less than is seen with typical MG positive sera. A test of their day-old progeny, which involved plate tests of 40 chicks, revealed no positive reactions. HI tests were conducted on the sera with positive plate reactions. The average HI titers were low and never reached the levels of 80 or 160 observed in typical outbreaks of MG. Isolation of MG from breeders was attempted on three occassions and was recovered only one time. In this instance MG was isolated from only one of eleven reactors cultured in December while M. gallinarum was isolated from nine of the eleven birds. MG was also isolated from six-to-eight-week-old broiler progeny of Flock A.

A similar persistent reactivity to the plate test, at a reduced level of intensity, and low HI titers were observed for flock B (Table 2). MG was also cultured from both the breeders and their progeny. The progeny had airsacculitis and had been submitted to the laboratory as a routine diagnostic consignment.

Standard USDA protocols were followed for plate and HI tests (1). As prescribed, four hemagglutinating units of USDA antigen were used in the HI tests. Salsbury MG antigen was used almost exclusively for the plate tests. Occasional comparisons with Burroughs-Wellcome and USDA plate antigens suggested that the former was less and the latter slightly more reactive than the Salsbury antigen.

Discussion

A persistent pattern of MG plate test reaction even though less intense than expected and despite low HI titers to the standard USDA HI antigens, is nevertheless, a strong indication of MG infection. This is particularly true when the persistent plate reactions exceed 10-20 percent of the serums tested. Additionally, negative plate tests of day-old chicks and negative cultural results at any single sampling of the breeders may be inadequate to rule out the presence of MG infection.

National Poultry Improvement Plan (NPIP) provisions for rating chicken breeding flocks for MG indicate that flocks with plate test reactions and negative HI tests could qualify for an MG clean rating (2). NPIP provisions also allow multiplier egg type breeding flocks to depend on cull chick MG plate tests as the main monitoring system to maintain an MG clean rating (2). This work indicates that

veterinarians and others responsible for providing professional advice and official ratings should stay fully informed of the MG plate testing pattern of breeder flocks in addition to HI titers of breeders and cull chick tests. Breeder flocks with persistent plate reactions, as described, should be cultured for mycoplasma by dependable methods and very carefully evaluated despite difficulty in gaining supplemental cultural or serological confirmation. Precise consistent identification of each individual flock throughout its official state or NPIP testing period is essential. Assignment of official flock numbers and use of a testing history record chart system should be helpful to official rating agencies.

In the course of this work, two apparent problem areas have been noted. First, antibodies for all strains of MG may not react equally to the standard USDA MG-HI antigen. Secondly, the presence of MG in broth and plate cultures may be masked by the overgrowth of other mycoplasma, most notably M. gallinarum. These two problems can make serologic and/or cultural confirmation of infection difficult and sometimes frustrating.

An official national reference service for primary isolation, typing, and identification of mycoplasma cultures is needed and would enhance consistency in serological and cultural evaluation of breeder flocks.

References

1. U.S. Department of Agriculture, Agricultural Research Service. Standard Methods for Testing Avian Sera for the Presence of Mycoplasma Gallisepticum Antibodies. Revised March 1966, 9 pp.

2. U.S. Department of Agriculture, Agricultural Research Service. The National Poultry Improvement Plan. 1975 ARS-NE-32-1.

Acknowledgments

The serological assistance of Mrs. Alyce Dugan, ASCP and reference mycoplasma isolation and identification services provided by Drs. S. H. Kleven, N. O. Olson, S. P. Sahu, G. Stein, Jr., and H. W. Yoder out-of-state and by Drs. N. B. Bowen and R. F. Gentry in-state are greatly appreciated. The generous advice and encouragement of Dr. J. Fabricant and Prof. O. M. Weinack is also very gratefully acknowledged.

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TABLE 1. Summary of laboratory reports of MG tests* for flock A housed in laying quarters June 1974.

Month	1974 F	M	A	M	J	J	A	S§	O	N	D	1975 J	F
Percent Plate Reactors Breeders	2%		8%		62%			39%		58%		29%	
Percent Plate Reactors Day Old Progeny												0%	
Average HI Titers (Breeders)					8			60		10			
Culture of Breeders											** Pos. for MG	Neg.	Neg.
Culture of 6-8 week progeny													Pos. for MG

* A total of 608 MG Plate (Sal) and 68 MG-HI (USDA) tests.

** M. gallisepticum (MG) isolated from 1 of 11 November reactors cultured in December. M. gallinarum isolated from nine of the other ten.

§ 120 sera from 2 flocks of 8 week old broiler progeny hatched in September were negative for MG and MS on plate test. Both flocks recovered only slowly from standard vaccination.

TABLE 2. Summary of laboratory reports of MG tests* for flock B housed in laying quarters November 1973.

	1973				1974						
	S	O	N	D	J	F	M	A	M	J	J
Percent Plate Reactors Breeders	0%	0%	43%	8%		66%		77%**	72%	40%	
Percent Plate Reactors Day Old Progeny							8%				
Average HI Titers (Breeders)			0	26		50		10	4	5	0
Culture of Breeders									Neg.		Pos. for MG
Culture of 6-8 week progeny										Pos. for MG	

* A total of 389 Plate (Sal) and 36 MG-HI (USDA) tests.

** Plate tests on paired sera by a private laboratory using MG plate (Bur-Wel) antigen revealed only 8% positive.

Mycoplasma Testing Problems in Primary Broiler Breeders

M. N. Frazier, D.V.M.

Introduction. The broiler breeder industry and the primary breeder in particular has been plaqued with problems involving the routine serology for control of the two major mycoplasmoses of concern to the broiler industry. Although the overall problem of respiratory disease and condemnations directly related to mycoplasmosis is greatly reduced in the past ten years, sporadic occurrences are still very costly to those involved.

Materials and Methods. The rapid serum plate (R.S.P.) tests in our laboratory are performed using the U.S.D.A. protocol. The Mycoplasma gallisepticum (M.G.) antigen is purchased from Salsbury Laboratories, Charles City, Iowa. Two Mycoplasma synoviae (M.S.) antigens are used. One is purchased from Salsbury Laboratories and the other from Intervet International B.V., Boxmeer, Holland, imported under permit from the U.S.D.A. In our hands the Intervet antigen appears to be adequately sensitive and more specific than the Salsbury antigen. U.S.D.A. produced serum plate antigen and antisera are used as controls.

The microtiter technique is used for the Hemagglutination Inhibition (H.I.) test using U.S.D.A. Hemagglutinating Antigen. The H.I. test for M.G. is performed using 4 H.A. units and for M.S. using 2 H.A. units. A titer of 1:80 or greater is considered positive.

Discussion and Results. The serological problems manifest themselves in several ways. The most common is probably the "transient false positive." In these instances the flock reacts to the R.S.P. test but is negative to the H.I. test. After a period of several weeks the reaction is greatly reduced or disappears altogether. The H.I. test results are negative during the entire period.

Another perplexing problem is the cross reaction between M.G. and M.S. This is almost entirely a one way cross reaction with a transitory M.G. R.S.P. reaction during the period of sero-conversion to M.S. infection. It is particularly frustrating because the cross reaction occurs to the M.G. antigen prior to any reaction to the M.S. antigen. One has to assume that this is due to a difference in the sensitivity of the antigens. The prolonged period before the appearance of H.I. antibodies to M.S. infection increases the time before a definitive diagnosis can be made on serology. Figure 1. shows the author's interpretation of the dynamics of the serology of M.S. infection. These conclusions are based primarily on observations, although some controlled investigations have been made.

Table 1. shows what the author considers to be a typical cross reaction between M.G. and M.S. occurring during the period that a flock is infected with M.S. and converting serologically from negative to positive. This type of problem, while not causing an economic disaster, does create havoc with state certification and hatching egg utilization, particularly if it is

prolonged several weeks, which is usually the case.

The possibility of a false negative creates far more apprehension than a false positive. A false positive reaction can usually be resolved and no lasting or irreparable harm is done. It is certainly costly and aggravating but not a calamity. A false negative can set in motion a chain of events that the repercussions are difficult to comprehend. Breeder pullet chicks could be delivered, in good faith, to a customer and concievably infiltrate the entire operation before detection. The possibility of complacency because of the high incidence of "false positives" is one of the most disturbing facets of the entire problem.

Tables 2, 3, and 4 give the serological results of a single flock at 1 week intervals for 3 weeks beginning at 47 weeks of age. These results were obtained in our laboratory following the report of a suspicious test result at a state laboratory. In the author's opinion these results are typical of an M.G. outbreak in a breeder flock. During the entire period the state laboratory involved reported the flock as negative. Fortunately, they did alert us that there might be a potential problem.

If the poultry industry is going to be able to solve other disease problems some of the aspects of the serology of the mycoplasmoses will have to be resolved, so that our efforts can be diverted to other endemic or emerging problems.

DYNAMICS OF M.S. SEROLOGY

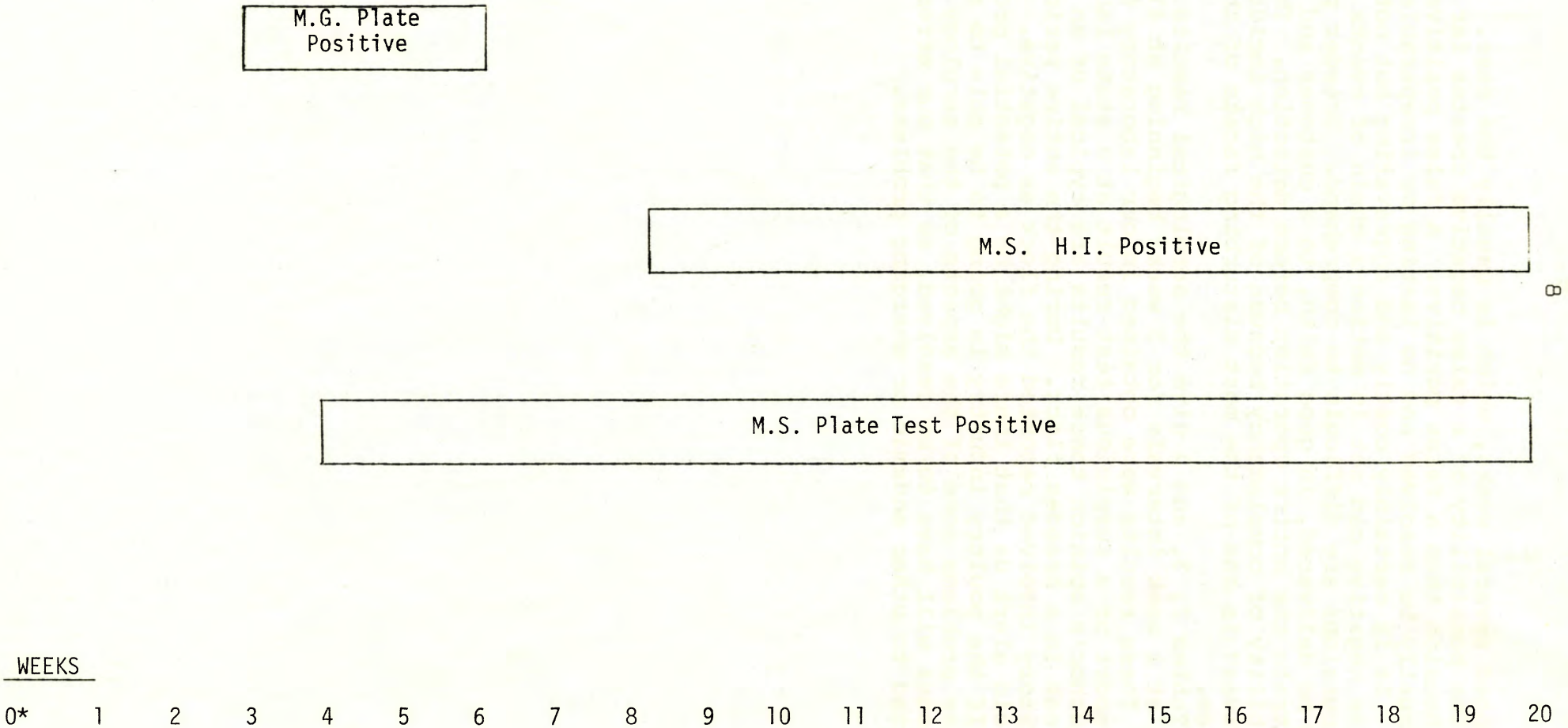

TABLE 1

MYCOPLASMA SYNOVIAE

An M.S. infected flock showing the cross reaction between M.G. and M.S. on the serum plate test. Any reaction on the plate, however slight, is recorded as a positive.

Date of Tests		House 1	House 2	House 3		House 1	House 2	House 3
10/25	M.G. Plate	33/160			M.S. Plate	0/160		
	M.G. H.I.	0/33						
11/07	M.G. Plate	1/83	26/92	23/95	M.S. Plate	0/83	70/93	46/95
	M.G. H.I.	0/1	0/26	0/23	M.S. H.I.		0/9	0/13
11/13	M.G. Plate	6/160	15/160	13/160	M.S. Plate	23/160	141/160	157/160
	M.G. H.I.	0/6	0/15	0/13	M.S. H.I.	0/5	0/10	0/8
11/27	M.G. Plate	4/80	8/90	5/70	M.S. Plate	41/80	88/90	49/70
	M.G. H.I.	0/4	0/8	0/5	M.S. H.I.	2/11	5/12	9/11
12/20	M.G. Plate	3/80	5/80	5/81	M.S. Plate	80/80	40/40	40/40
	M.G. H.I.	0/3	0/5	0/5	M.S. H.I.	4/4	3/3	3/3

TABLE 2

47 WEEKS OF AGE

Mycoplasma gallisepticum

Serum plate	50/50
H.I. test	3/10 (1:160;1:160;1:80)

Results of serum plate and H.I. (Microtiter) tests at the time the flock was termed suspicious.

TABLE 3

Mycoplasma gallisepticum

Bird No.	48 weeks of age	49 weeks of age
1	1:640	1:320
2	1:320	1:640
3	1:40	1:160
4	1:160	1:320
5	1:160	1:160
6	1:40	1:320
7	Neg.	1:40
8	Neg.	1:320
9	1:40	1:80
Total positive	4/9	8/9

H.I. test results on individual banded birds. Flock was classified as suspicious.

TABLE 4

49 Weeks of Age

Mycoplasma gallisepticum serum plate test 200/200

M.G. H.I. test results on 30 birds

	Microtiter (plate)	Macrotiter (tube)
1:640	3	0
1:320	1	1
1:160	6	4
1:80	9	13
1:40	8	7
1:20	2	5
1:10	1	0
	30	30
Total number at 1:80 or greater	19/30	18/30

Comparison of H.I. tests using the Microtiter and the Macrotiter systems. Flock was still classified as suspicious.

ANTIGENIC VARIATION AMONG ISOLATES OF MYCOPLASMA GALLISEPTICUM.

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Traditionally, the diagnosis of avian mycoplasma infections has been based, in part, on serology. The serum plate agglutination (or tube agglutination) test has been used to screen turkey and chicken flocks for M. gallisepticum (MG) and M. synoviae (MS) and turkey flocks for M. meleagridis (MM). Suspicious reactions are then confirmed by the hemagglutination-inhibition (HI) test. This procedure has been extremely useful in the detection of infected flocks.

Unfortunately, recent experiences have indicated that the serological diagnosis may be somewhat more complex, especially in chicken flocks. One problem has been the presence of nonspecific agglutinins in certain flocks; occasionally a few HI titers from these birds may be in the suspicious to positive range. These reactions may disappear spontaneously or persist for long periods of time; however, the flocks are negative on the basis of mycoplasma isolations.

Another problem has been outbreaks of "avirulent" MG infection in flocks 30 weeks of age or older. Typically, it takes weeks or months for a high proportion of reactors to be detected - the flock may never have over 40-60% reactors on the serum plate test. The HI titers develop extremely slowly, and rarely exceed 1:80 to 1:160 and these are present in only a small number of birds.

In these flocks MG can be isolated from tracheal swabs if one is very persistent. Unfortunately, the confirmation of the diagnosis may take weeks to months.

Recently, MG isolates have been made from a few breeder flocks exhibiting a low percentage of birds with agglutination reactions. However, in these cases, HI titers were never high enough to incriminate the flock as being MG-infected. Isolates of MG have been made from several of these flocks, but are considered to be "atypical" or antigenic variants. The isolates have typical biochemical reactions for MG. Growth is not inhibited by antisera against standard strains, and the FA tests are only weakly positive.

The MG variants, when used to infect experimental birds, elicit a typical serum plate agglutination response which is detectable with standard antigens. However, only negative or very low HI titers are detected with standard antigens. HI antigens made from the "variant" isolates are likewise unable to detect titers elicited from a "standard" MG infection. In both cases, diagnostic HI titers were detected only with the homologous antigen. Thus, MG isolates all cross react on the agglutination test, but considerable strain variation may occur with the HI test.

These results do not negate the value of the HI test - especially when it is positive. The presence of persistent agglutinins with negative or low HI titers should lead one to suspect an early infection or infection with an "atypical" MG isolate. These cases may be resolved only by isolation and identification of the causative agent.

Further Studies on Field Isolates of Avian Mycoplasma

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Abstract

During the past 5 years more than 40 flocks of chickens have been studied to determine their Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) status following preliminary serological tests reported as suspicious or positive reactors by various state or private laboratories. Such flocks have rarely evidenced clinical signs, have usually been 26 weeks of age or older, and usually contain a relatively small percentage of weak to moderate agglutination reactors. Hemagglutination-inhibition (HI) titers have usually been too low to be definitely positive. Repeated testing at 14 day intervals has often failed to provide information for proper interpretation.

MG or MS separately can usually be isolated and identified during repeated cultural studies from flocks if the moderate HI reactions linger or elevate, though the percentage of reactor birds may never become greater than 20-30%. No proof of mycoplasma infection has been obtained from "transient reactor" flocks even though the moderate reactions may have lingered for 3 or 4 months before regressing.

Results of studies on numerous isolates of mycoplasma from the described flocks are as follows:

1. MS cultures tend to be present in a large percentage of MS infected birds within a given flock even though initial reactor rates may be low.
2. The MS isolates from such flocks do not seem to vary a great deal from the usual MS cultures.
3. The MG cultures tend to be very difficult to isolate and grow relatively slow. Often 20 to 40 birds must be cultured to find one or two isolates per flock.
4. These MG isolates tend to be representative of the genus by some serotyping procedures. However, their relative antigenicity in inoculated chickens may actually vary with their possibly reduced virulence or pathogenicity.
5. The HI titers produced in chickens inoculated with these newer MG isolates tend to increase with more extensive challenge such as aerosol exposure plus simultaneous infection with infectious bronchitis virus.
6. The ability of these MG isolates to produce obvious airsacculitis in broilers predisposed by infectious bronchitis infection is often about the same or a little less than MG isolates designated as typical pathogens.
7. The true significance of these newer MG isolates is not

readily established. However, it is clear that when MG is isolated and identified from a flock with questionable serology that flock must be considered MG positive.

I personally feel that most recent mycoplasma problem flocks have yielded either MG or MS of somewhat less virulence or less pathogenicity, thus they invade slower, spread slower, and truly stimulate weaker serological reactions. However, these same cultures tend to stimulate sufficient MG or MS antibodies in inoculated chicks to readily afford confirmatory culture typing serology.

One possible partial exception to this idea is that forwarded by Dr. Kleven that some of these cultures evoke far better HI titers with homologous antigens than with "standard" HA antigens. I think that Dr. Frank Craig has promoted this idea for several years also. I can accept the idea, but have no evidence to prove its practicality. However, we have some studies in progress aimed at they very aspect. If research proves that one or more special selected HA antigens can truly yield significant HI titers in otherwise questionable reactor flocks, we might then be able to avoid the costly slow culture studies we now feel are so important.

The single most obvious problem at hand is just exactly how to diagnose MG and MS reactor flocks. Some new or adapted serological procedure must be devised for practical use. However, we still need ample cultural studies during this developmental work.

Evaluation of Flocks for Nonspecific *Mycoplasma synoviae* Reactors by the Use of Isolation and Serum Plate Agglutination, Hemagglutination-Inhibition and Agar Gel Precipitin Tests

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Abstract

Serums from 65 broiler breeder flocks were evaluated for *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG) antibodies by serum plate agglutination (SPA), micro hemagglutination-inhibition (HI) and agar gel precipitin (AGP) tests. The nonspecific SPA reaction in serum from breeder flocks was eliminated by diluting the serums 1:5 in phosphate buffered saline (PBS) pH 7.2 before testing provided the MS antigen reacts with 1:10 dilution of the MS anti-serums. The nonspecific SPA reaction was confirmed by negative HI and AGP reactions. When specific MS reactions occurred the serums showed a micro HI titer of 1:80 or greater, an AGP line and MS was isolated from the upper respiratory tract of infected birds. Serums from experimental birds on Aureomycin^{R}, 200 g/ton of feed, were positive on SPA test but were negative for micro HI and AGP tests. MS was isolated from these birds. Nonspecific SPA MG reaction occurred in many of the flocks which were negative by MG HI and AGP tests.

If MS is isolated from a flock it is very conclusive, but if it is not isolated further tests may be needed. We are of the opinion that if a flock is positive by 2 out of the 3 following tests, namely, SPA, AGP or HI tests, the flock should be considered positive. The three tests mentioned above are flock tests and should be made on a large number of samples to represent the flock. In our screening procedures, 100% of the samples received (70-100) were tested by the SPA and micro AGP tests. The micro HI test was conducted on 20 or 20% of the samples.

Animal and Plant Health Inspection Service

Activities in Light of Recent Mycoplasmosis Findings

Claude J. Pfow

Our Agency (APHIS), with the guidance of several research scientists, developed the protocol for the mycoplasma antigens in the early 1960's that are now being used by commerical producers.

After turning the protocol over to the commerical laboratories, APHIS has continued to produce reference antigens and serums to be used as controls in testing laboratories. We also continued training sessions for laboratory technicians aimed at encouraging uniformity within the testing laboratories. Because there is no commerical source for the HA antigen for Mycoplasma gallisepticum and M. synoviae, we have produced and distributed them to testing laboratories on request from the State animal health officials.

Unfortunately, during the past few years other program priorities have kept our manpower and budget to the level that we have had to forego any developmental work on antigen production in order to supply the laboratories with reference material.

We plan, however, to continue distributing reference materials on request at approximately the same levels as last year during fiscal year 1976.

APHIS has conducted limited field studies in the past few years in cooperation with primary breeders and State testing laboratories, and it was learned that there are some differences in antigens and some variations between testing laboratories in qualifying breeder flocks. The problem goes far deeper than that however. There are instances when flocks that were negative when 100 percent tested, later showed positive reactions and even transmitted to their progeny when there was no obvious way these breeders could have become infected.

There is no question that we are facing serious problems in recognizing mycoplasma infections in breeder flocks. It appears that the most acute problem involves the identification of clean or infected flocks at the time of testing.

At one time we relied heavily on the HI test to sort out questionable reactions. Quite often now, the HI test is of little value in identifying the true status of a breeder flock.

In response to numerous requests from the poultry industry, APHIS has agreed to undertake limited field studies of problem breeding flocks. Because of limited funds and personnel it was decided that two of our regional epidemiologists would each spend approximately 7-1/2 percent of his time on this project. This would limit each of them to work with only one breeder flock at a time.

Early in May 1975, APHIS personnel met with the Chairman of the Mycoplasmosis Committee of the American Association of Avian Pathologists to come up with a unified plan.

The agreement was that one epidemiologist would select a chicken breeder flock, the other a turkey breeder flock in his region that was having unusual serological responses that, hopefully, would go

through a production cycle. It will be necessary to have full support and cooperation of the owner.

It was determined that a different laboratory would furnish support for each epidemiologist but the techniques used in each laboratory would be as near the same as possible. These techniques would be those recommended by the Mycoplasmosis Committee of the AAAP; namely:

1. In chicken flocks reacting to the official serological tests for MG and MS which cannot be clearly defined as positive or negative by these tests, cultural procedures should be carried out.
2. This committee suggests that samples of a minimum of 100 tracheal swabs should be collected and cultured for each of these organisms by the techniques described in pages 217-220 of the "Methods for Examining Poultry Biologics and for Identifying the Quantifying Avian Pathogens."
3. These swabs should be collected, put directly into liquid media and transported to the laboratory. They are then incubated for 3 days at 37°C and plated onto solid media. The agar plates are incubated in a humid atmosphere for 6 days and examined for colonies.
4. When mycoplasma colonies are seen they must be identified as either MG or MS by adequate serological procedures. The quickest and simplest of these is fluorescent antibody staining of colonies directly on agar blocks or agar discs.
5. The most efficient media for isolating these two mycoplasma species are Frey's Modified French Media with and without NAD or Fabricant's BS-BA Media with and without NAD.

FLOCK NUMBER ONE

This flock is a primary breeder flock of leghorns selected by Dr. D. C. Johnson in the South East Region. The flock was brooded in September 1974, and on routine blood testing; the serological responses were as follows:

1/15/75	21 MG pos	293 MS pos
	7,599 tested	460 tested
	12 MG reactors had HI titers 1:20	
4/10/75	28 MG pos	0 MS
	492 tested	50 tested
	MG reactors had HI titers 1:20	
5/14/75	142 MG pos	No MS tests
	500 tested	

Neither M. gallisepticum or M. synoviae was isolated, so the flock was cleared, housed, and is in production. In depth cultural and serological studies will be made on the flock and its progeny in order to get all the data possible from it. Five mycoplasma isolations have been cultured but not typed. They are not typical MG or MS.

Dr. Harry Yoder at the Southeast Poultry Research Laboratory, Athens, Georgia, is furnishing the laboratory support with back-up support from Dr. Walter Martin at our Veterinary Services Laboratories, Beltsville, Maryland.

FLOCK NUMBER TWO

Dr. Dyarl King was unable to locate a turkey breeder flock in the North Central Region that was exhibiting unusual serological responses. A grow-out turkey flock, however, was found that had symptoms of acute airsacculitis at 5 weeks of age. An isolation of an unidentified mycoplasma was made from tracheal swab cultures from the flock. The isolate is being further studied at Veterinary Services Laboratories, Ames, Iowa. Random selected birds from the flock will be culturally and serologically observed throughout the grow-out period and at time of slaughter.

Traceback efforts to determine transmission from the parent flocks are being carried out. Dr. Billie Blackburn at VSL, Ames, Iowa, is furnishing laboratory support for this flock.

Dr. Walter Martin at VSL, Beltsville, has developed an HA antigen from "Klevin's 503" isolate that will be used in future serological studies in problem flocks. APHIS does not plan to distribute this antigen to testing laboratories at this time. Other "variant" isolates will be studied as they are identified in the future.

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Memorandum

SUBJECT: Reply to Mycoplasma Committee Memorandum

DATE: October 23, 1975

TO : Dr. Frank J. Siccardi
2850 Inwood Lane
Fayetteville, Arkansas 72701

As you know, Dr. Dyarl King and myself are working with mycoplasma problem flocks. The flock I have been working with and now being terminated would give any laboratory difficulty in determining if it was free from infection. It took 5 months from the date of the first suspicious reactions to successfully culture MG. Numerous attempts were made from 91 birds before a slow growing, difficult to characterize MG was isolated from a 41 week old bird submitted to the laboratory as an MS reactor. Numerous MG isolations have since been made from this flock. A sister flock is being studied at the present time.

Following are some important questions I think the committee needs to consider.

1. What constitutes sufficient culturing of suspicious birds to declare a flock free?

2. Should a flock be declared free when it contains suspicious reactors?

3. Are all of the atypical MG isolates pathogenic?

4. What causes the transient MS reactions when no MS is present?

Culturing of a difficult to isolate Mycoplasma that takes 6 months does not meet the diagnostic needs of the breeder flock owner. We need some better diagnostic tools. Obviously some additional research is needed to give us some quicker answers.

Daryl C. Johnson

Daryl C. Johnson
Regional Poultry Epidemiologist, Southeast

Proposed Position Paper on Controlled Exposure to *Mycoplasma gallisepticum* in Commercial Egg Operations

Dr. Siccardi appointed a sub-committee of the AAAP Mycoplasma Committee to draft a position paper re the willful exposure of replacement laying chickens to *Mycoplasma gallisepticum*. He charged that sub-committee with the development of facts on which to base the paper. Let me make it crystal clear that in all of this discussion *M. gallisepticum* is the only organism being discussed. It was also strongly suggested that as an organization the AAAP should categorically condemn this practice. With one exception, the replies received to a letter sent members of the sub-committee and others (and not everyone answered) avoided the categorical condemnation of the practice.

Opinions expressed were based on observations, relatively few on controlled trials. There was total unanimity in the feeling, and it was very strongly expressed in almost every instance, that the goal should be total MG eradication. The road to that goal is not quite so clear.

It is very apparent that there is no agreement on the precise damage that conversion from negative to positive status in a commercial laying flock is uniform in its effect. A great deal depends on the presence of other infections, environmental stresses or managemental slip-ups. There was never any question in any comments, oral or written, that willful exposure should be practiced for birds used for hatching egg production. This is a very definite "no no". There seemed little question about the value of producing chicks and growing them free of *M. gallisepticum* infection. This was universally desired by all, even though some favor the conversion of those birds (to positive) prior to the initiation of lay.

Here are some quotes:

"The Board of Directors of the Southeast Poultry and Egg Association, almost equally divided as to broiler or commercial egg production, voted unanimously to oppose any program calling for controlled exposure to Mycoplasma infections. Our position can be simply stated in one word--No."

Only half facetiously I would ask whether the individuals voting were "all in all out" and whether they voted and act alike.

Another quote:

"I believe pullets can be reared with many fewer complications if they are MG negative. Secondly, if they are later placed on lay ranches where they probably will be exposed to MG, the transition from negative to positive status will be sub-clinical unless complicated by coryza. In cases where exposure to both MG and coryza are likely to occur after delivery to the laying area, I would probably expose them to a mild strain of MG such as the 'F' strain during the latter part of the rearing stage to prevent the serious complications of a dual exposure."

This last statement was based on observations from a large multiple age ranch and was backed up with data that can be presented if time permits.

Another quote:
"Some of the dangers I see in this practice of exposing pullets to MG, if followed to any large extent are 1) Danger of spread to other species of poultry. While some of the strains are innocuous to chickens their effect on other species may not be known. 2) Unless care is taken vaccines could be contaminated by the producing laboratory. 3) Conversely, other agents could be spread by the MG vaccine. 4) There is little hope of eradicating MG from the poultry if we persist in spreading it."
Another states:
"I would be in favor, therefore, of a strong recommendation against controlled exposure of any breeding flock but controlled exposure of egg-laying replacement stock to MG during the growing period may be a recommended practice under certain circumstances."

By way of comment, I think there's good evidence to indicate that "all in all out" MG-free flocks produce 2 to 10% more eggs per year than do multiple egg operations. In addition, pullets set down, day-old, free of MG have many less problems during the growing stages and through routine vaccination practices than do those that are infected. As one of the respondents indicated: "Since we have brooded approximately 2 1/2 million pullets and we have not used any medication (with MG-free pullets at day of age)".

One veterinarian made this statement: "The broiler and turkey meat producing industries are virtually MG-free and have remained free in spite of widespread MG infection in commercial layers and backyard poultry. Thus, MG infection in some layer populations presents only a minor hazard to MG-free chicken and turkey populations, provided sound management practices are followed." In this connection, another veterinarian for a basic broiler breeder, indicating I could quote him, stated that having basic breeding stock and extremely tight security they were able to maintain MG freedom despite widespread willful exposure in their state, Connecticut. He expressed the concern, however, that in those areas where multiplier flocks are kept and security may not be as consistent or as tight there might be greater danger of spread in the use of deliberate infection for replacement layers. One of the comments also stated that firsthand experiences with willful infections had demonstrated that this procedure does not always circumvent the complicating infections (coryza, LT, etc.), alleviate environmental stresses or insure that laying performance on infected lay ranches will be satisfactory.

There's widespread opinion that was expressed that the decision of exposing pullets or cleaning up lay ranches will be made on the basis of economics, and that therefore the AAAP should take the positive approach of emphasizing the hazards of exposure, the advantages of clean stock and provide guidance and leadership in the most effective use of willful infection rather than categorically being against it as we are against sin. Yet most of us will take a cocktail before dinner!

There's a feeling that those wishing to improve lay ranch performance through pullet exposure during the grow period should be encouraged to delegate supervision and planning of the program to veterinarians with expertise in avian pathology. Another quote:
"Since exposure will be attempted, a satisfactory strain of MG should be made available to veterinarians supervising these operations."

Most of you may not recall the widespread transfer of water and feathers and birds to insure that replacement layers would become infected with mild Newcastle disease before vaccine was available, with infectious bronchitis before the birds came into lay, also before vaccine was available.

From a turkey representative: "The only way I can see the use of MG culture in chickens is on a permit basis by the state disease control agency. I can appreciate the reasoning back of the use of live culture in some establishments, but it certainly must be controlled to assure the purity, safety and efficacy of the product and to control the indiscriminate use of the product since it will not be produced under a license." He goes on to say: "A laboratory that produced the vaccine should be qualified on a permit basis in order to control the quality of the product and this would be up to each state." A further comment from the same individual: "Thus, I am opposed to the official sanction of the controlled exposure program by AAAP. The AAAP Mycoplasma Committee should go on record in opposition to the program."

Apparently, also, the Poultry Health Committee of the Poultry and Egg Institute, which I didn't see, came out in opposition to the use of controlled exposure program on replacement pullets.

That controlled exposure, or as it once was stated, uncontrolled exposure, does jeopardize the free flocks is very true, particularly if it is done randomly, with cultures and materials not either well standardized or known to be effective. A southern California veterinarian says:

> "In summary, combined infections with MG, Hemophilus, and Pasteurella of various species produces a very serious chronic respiratory disease, resulting in poor production and high lay mortality. In southern California more than 95% of the egg laying flocks carry these infections. If growing pullets raised in isolation are infected with MG at 8 to 10 weeks and delivered at 20 weeks, completely vaccinated, no serious problems occur when they are placed on a ranch where these infectious occur. On the other hand if pullets are delivered MG-free to a ranch infected with MG, HG and Pasteurella sp., serious problems develop. Poultrymen who have experienced these problems will not accept MG-free pullets."

There are problems in designing a program, particularly if pullet producers grow birds for ranches which are MG-free and wish to remain so and concurrently produce other broods for ranches which are infected and which demand MG-positive birds. Quite obviously, on the same premise this poses grave problems, some total rearrangement by the pullet grower and the two classes of customers would be necessary. It seems even more unrealistic to think that the same pullet producer on the same premise and with the same employees can expose chickens to M. gallisepticum on the one hand and maintain a free group on the other. Most unsuccessful attempts to clean up populations from MG have been the result of breakdowns in security and management and particularly the transfer of infection through personnel moving indiscriminately between infected and uninfected groups of birds.

Here is a succinct comment:

"We serve an industry that can survive only by choosing effective procedures. To tell that industry it can't use a procedure because it is esthetically undesirable or professional frowned on is patently ridiculous. I refuse to be deprived of a treatment that may solve someone's problems. Professionally I'll decide whether someone else is placed at risk. Does the association think it can stop a practice if it is beneficial? I point to Marek's protection! What is the difference between MG exposure and AE exposure? They are technically and biologically used to prevent problems in specific situations. Coryza exposure is less satisfactory from the point of view of results than is MG exposure--still we have the choice of telling a man to use it or close his business. My experience has taught me that MG freedom is economically very valuable and I support the goal of MG eradication. MG eradication is preferable to MG protection and both are preferable to MG infection in maturing pullets. For our clients, the poultry industry, let's encourage experimentation, gather the evidence, present it in its most useful way for the individual poultryman's decision. When that decision is MG exposure, as it most certainly will be in some cases, than we point out how to do it and how to ultimately stop doing it".

In summary, I should like to propose as a resolution to be acted upon by the general meeting tomorrow, the following:

WHEREAS, the benefits of complete freedom from Mycoplasma gallisepticum infection have been well documented and are generally accepted and

WHEREAS, the maintenance of freedom from the infection of those populations which are demands careful surveillance and high security and

WHEREAS, uncomplicated Mycoplasma gallisepticum infection can occur either prior to production or even in some cases during production with no proven severe detriment and

WHEREAS, Mycoplasma gallisepticum complicated by other infections or stresses and occurring by transfer in multiple age ranches from infected to clean brids can cause serious economic damage and

WHEREAS, the American Association of Avian Pathologists recognizes the diversity of needs as well as opinions and the desirability of accumulating increased information, therefore

BE IT RESOLVED that the American Association of Avian Pathologists strongly endorse continuing emphasis on the desirability of attaining and retaining freedom from Mycoplasma gallisepticum and be it further resolved that because of economic needs as a stop-gap measure, exposure of certain growing pullets may be economically necessary that the Association encourages further investigation and supervision of any such deliberate preventive infection measure by qualified public or private personnel, and

BE IT FURTHER RESOLVED that standardized cultures be made available on a permit or supervised basis in order to do the most effective job possible when exposure seems the only sound economic and veterinary alternative.

A. S. Rosenwald, D.V.M.
Chairman, Sub-Committee

Veterinarians consulted--not all replied:

Dr. Walter Hughes
Dr. Don Zander
Dr. Reed Rumsey
Dr. Stanley Vezey
Dr. Ben Pomeroy
Dr. John Allen
Dr. Earl Grass
Dr. Dave Clark
Dr. Everett Bryant
Dr. William Dungan
Dr. Julius Fabricant
Dr. Harry Yoder
Dr. Lee Grumbles
Dr. Frank Siccardi
Dr. Phil Levine
Dr. Irvin Peterson
Dr. John Walker
Dr. Monte Frazier

Standard Cultural and Serological Procedures for the Diagnosis of Avian Mycoplasma Infections

Recommendations of AAAP Subcommittee on Mycoplasmosis
St. Paul, Minnesota
June 12, 1975

In chicken flocks reacting to the official serological tests for MG or MS which cannot be clearly defined as positive or negative by these tests, cultural procedures should be carried out, in addition to repeat serological tests.

This committee suggests that samples of a minimum of 100 tracheal swabs should be collected and cultured for each of these organisms by the techniques described in pages 217-220 of the "Methods for examining poultry biologics and for identifying and quantifying avian pathogens."

These swabs should be collected, put directly into liquid media and transported to the laboratory. They are then incubated for 3 days at 37°C and plated onto solid media. The agar plates are incubated in a humid atmosphere for 6 days and examined for colonies with a dissecting microscope at least 20 X magnification, preferably under oblique transmitted illumination.

When mycoplasma colonies are seen they must be identified as either MG or MS by adequate serological procedures. The quickest and simplest of these is fluorescent antibody staining of colonies directly on agar blocks or agar discs.

The most efficient media for isolating these two mycoplasma species are Frey's modified French medium with and without NAD or Fabricants BS-BA media with and without NAD.

The following items were also emphasized:

1. There is an acute need for a central mycoplasma identification center.
2. A commercially available source of serotyping reagents, such as immunofluorescent conjugates, is needed.
3. The use of micro HI systems is encouraged because of the relatively limited supply of antigens.
4. A subcommittee of the mycoplasma committee should be utilized to evaluate the specificity of FA conjugates.
5. The widespread distribution of HI antigens from "varient" MG isolates is not recommended, but further research on this problem is needed and encouraged.
6. The subcommittee encourages the continued supply of quality antigens from commercial sources and continued production of USDA standard reagents.
7. Research on the improvement of serological tests and on the determination of the cause(s) of serological tests should be encouraged.

TURKEYS

Turkeys may be naturally infected with M. gallisepticum, M. synoviae, M. meleagridis. Currently the NPIP is only concerned with an official program for MG. However, serologically and microbiological programs for MM and MS are suggested for primary breeder flocks that may wish to be identified as clean of these infections.

M. gallisepticum (MG) Qualifying Requirements

1. Initial Test - (Minimum) (SPT or TT)
   10% of Flock or not less than 300 birds or 100% test of flocks less than 300 birds (16 weeks of age or older).
2. Flock is considered Qualifying for U.S. M.G. Clean Classification when all serological tests are considered negative.
3. Flock is considered suspicious or positive under the following circumstances:
   a. Sera giving any serological reactions (Susp. and Pos.) be retested by HI test (Macrohemagglutination Inhibition Test (Tube)). At the present time the micro HI test has not been standardized for turkey sera.
   b. Interpretation of HI test. Any reactions 1-40 or higher be considered suspicious for M.G. infection.
   c. Classification of Suspicious flock.

Before a flock is considered infected the following criteria should be satisfied:

i. At least 5 birds giving suspicious reactions be submitted to the laboratory - The serological tests be reconducted on the individual birds.
ii. Birds necropsied and examined for lesions.
iii. Attempts be made to isolate M.G. from sinus exudate, trachea and air sacs.
iv. If Mycoplasma is isolated the organism must be serotyped by Fluorescent Antibody Technique (FAT) or Growth Inhibition Test (GIT) or bird inoculation (SPF chicken or turkey) accompanied by positive serological response for MG. If the Mycoplasma is serotyped as MG, the flock is considered infected.
v. If the serological response for MG is suspicious and no isolation of MG is made and lesions suggestive of MG are observed, then precautions should be taken to eliminate MS. In addition a visit to the flock should be made by a representative of Official Animal Health Control Agency or Official State Agency.

It may be necessary to conduct serological and cultural examinations. This may include taking 100 tracheal swabs and blood samples from the same birds.

If the serology is suspicious for MG (SPT or TT plus HI test) and MS serology is negative and signs and lesions suggestive of MS are found, the flock should be considered infected with MG.

If MG is isolated along with serological response, the flock is considered positive.

If the serology is suspicious for MG and MS and neither organism is isolated, the flock should be retested by serological and cultural methods and considered suspicious until proven negative or positive for MG, or MS or both.

M. synoviae (MS) Qualifying Requirements

Since MS will produce sinusitis, airsacculitis and synovitis or any combination of these signs and lesion, the primary breeder or multiplier may wish to determine if a flock is free of MS infection.

1. Initial test
   a. Initial test may be conducted at the same time as MG test using SP or TT antigen. If any reactions to the SP or TT occur, the sera are tested with HI test (Macrohemagglutination-Inhibition test.)
   b. If the serological tests are negative, the flock must be retested at 28-30 weeks of age or at the time of first insemination. If the retest is negative, the flock may be considered negative. It is suggested that a third test be conducted at midpoint of production or at marketing or at any time the flock shows signs of respiratory infection and or lameness.
2. Interpretation of HI test.
   Any reaction of 1:20 or higher is considered suspicious for MS infection.
3. Classification of Suspicious Flock.

Before a flock is considered infected the following criteria should be satisfied:

i. At least 100 tracheal swabs and blood samples should be submitted to the laboratory for serological and cultural examination.
ii. If a Mycoplasma is isolated, the organism must be serotyped by FAT, or GIT or bird inoculation. If the Mycoplasma is serotyped as MS, the flock is considered infected.
iii. If MS is not isolated and serological tests are suspicious, and there are clinical signs and lesions of MS, the flock is considered infected.
iv. If MS is not isolated, and there are no signs and lesions of MS, and the serology is suspicious, the flock should be examined serologically and culturally for MS until proven negative or positive.

M. meleagridis (MM) Qualifying Requirements

The primary breeder or multiplier may wish to determine if a flock is free of MM infection. The following criteria are suggested to identify a flock free of MM.

1. Initial Test or Retests (SP or TT)
   a. The initial test may be conducted at the same time as MG test using SP, TT or Microagglutination test (MA). If any reactions to the SP or TT or MA occur, the sera are tested by HI test (Macrohemagglutination-Inhibition test).
   b. If the serological tests are negative, the flock must be retested at 28-30 weeks of age or at the time of the first insemination. If the retest is

negative, the flock may be considered negative. It is suggested that a third test be conducted at midpoint of production or at marketing or at any time the flock shows respiratory signs.

2. Interpretation of MM HI test.

Any reaction of 1:20 or higher is considered suspicious for MM infection.

3. Classification of suspicious flock.

Before a flock is considered infected the following criteria should be satisfied:

i. At least 100 tracheal swabs and blood samples should be submitted to the laboratory for serological and cultural examination.

ii. If a Mycoplasma is isolated, the organism must be serotyped by FAT, or GIT, or bird inoculation. If the Mycoplasma is serotyped as MM, the flock is considered infected.

iii. If MM is not isolated and serological tests are suspicious, the flock should be re-examined serologically and culturally until proven positive or negative.

Characteristics and Evaluation of Screening Tests

R. Yamamoto

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The Mycoplasma gallisepticum (MG) rapid plate test is an example of a screening test. A screening test in itself is not diagnostic but it merely attempts to tell us whether or not an individual (or flock) in question is infected and/or diseased.* One thus speaks of the sensitivity and specificity of a test in reference to its accuracy in discriminating between infected and noninfected animals (or flocks). A test is considered highly sensitive if it is able to identify a high percentage of infected individuals of the infected population tested (yields very few false negatives). Conversely, a highly specific test is able to identify a high percentage of non-infected individuals of the noninfected population tested (yields very few false positives).

Evaluation of Screening Tests. The sensitivity and specificity of a screening test may be evaluated provided a true diagnosis is made on the entire population on which the screening is performed. This may be shown in a table of four-fold classification as follows:

Screening Test Results	True Diagnosis** Diseased	Nondiseased	Totals
+	a	b	a + b
-	c	d	c + d
Totals	a + c	b + d	a + b + c + d

**Example: Agglutination = Screening Test; Necropsy and Bact. Exam. = True Diagnosis.

a = Diseased individuals detected by screening test.
b = Nondiseased individuals detected by screening test.
c = Diseased individuals not detected by screening test.
d = Nondiseased individuals negative to the screening test.

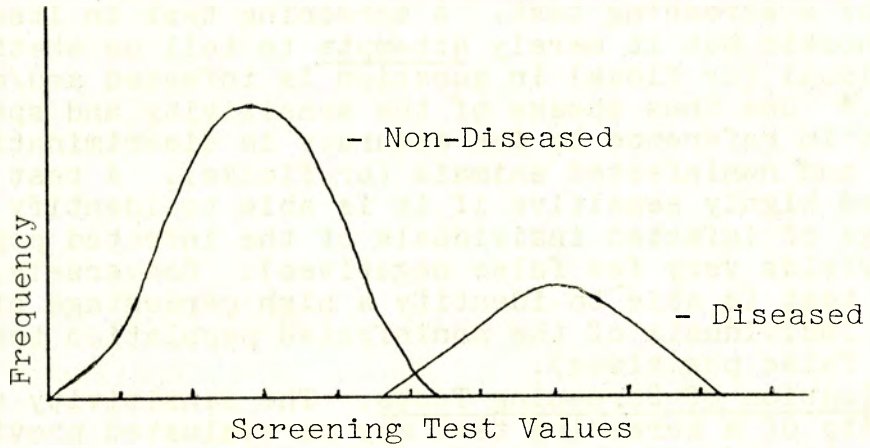
One raw data are accumulated, they may be analysed (see previous table) as follows:

$$\text{Sensitivity} = \frac{a}{a + c} \times 100 = \%\text{ of diseased individuals detected by screening test.}$$

$$\text{Specificity} = \frac{d}{b + d} \times 100 = \%\text{ of nondiseased individuals detected by screening test.}$$

*The terms, disease and infection, are used interchangeably in this paper.

Reasons for False Positives and Negatives. If one visualizes that the population to be tested consists of two subpopulations of diseased and nondiseased individuals, then the population may be graphically depicted on the basis of the "Theory of overlapping population distribution" as follows:

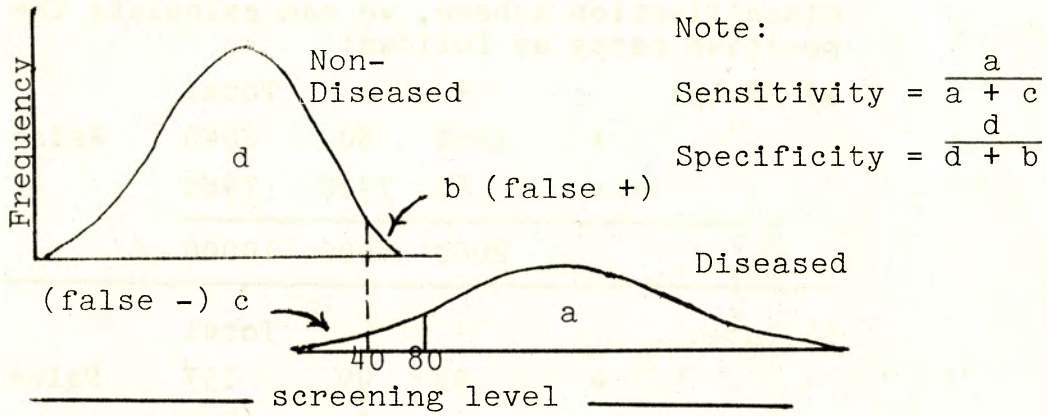


The above graph shows that the problem of false negatives and false positives arises because a certain proportion of the nondiseased population possesses attributes common to the diseased population and vice versa, i.e., the area of overlapping of the two populations. A screening test may be 100% sensitive and 100% specific only if the two populations do not overlap which is highly unlikely for most diseases.

Any factor which changes the shape of the curves and thus affect the overlapping area, will influence the sensitivity-specificity values of a test. These factors include age, sex, breed, geographic area, seasonal variation, and management practices. Thus, if one were to determine the sensitivity-specificity values of a test (e.g., MG agglutination) in one geographic area, the values may not be valid in another area if that area had a higher cross reaction rate (e.g., M. synoviae which cross reacts with MG may be more prevalent in one area than in another, and consequently, the specificity of the test may be quite poor in the area where M. synoviae is prevalent). These variations in the population should be recognized but they cannot be controlled.

Controlling Sensitivity and Specificity of Screening Tests. The sensitivity and specificity of a screening test may be increased by selection of a proper test(s) and by manipulation of reagents within a single test. For example, the selection of a proper antigen strain (by trial and error) could increase sensitivity, specificity, or both. Any serologic test may be manipulated within limits to increase or decrease its sensitivity-specificity, and includes such things as salt concentration, pH, antigen concentrations. As to the choice of the test, the MG agglutination test is more sensitive than the HI test since it detects antibodies much sooner after infection. The HI test may be more specific since it detects IgG, the antibody class which tends not to cross react with other antigens.

Screening Levels. Another commonly used method for changing the sensitivity-specificity of screening tests is to change the level at which a test is considered positive. For example, if we found that a 1:40 (i.e., 1:40 or greater) screening level of the HI test is detecting too many false positives, we may wish to change the screening level to 1:80 which should eliminate many false positives. Such manipulations are shown graphically in the figure below:



Unfortunately, any manipulation which increases sensitivity will decrease specificity and vice versa. For example, if we decide to interpret all transient plate reactors as negative, we will undoubtedly increase specificity but will be decreasing sensitivity at the same time. Therefore, a combination of approaches (or test procedures) may be necessary to solve this problem most efficiently.

Apparent Increase in False Positives During Late Phase of Control Program. During the early phases of a disease control program, the proportion of diseased animals in the population is relatively high. As the program progresses with removal of infected animals (and flocks), the population becomes essentially non-diseased. Assuming the the sensitivity-specificity values of a test remain constant (i.e., no changes in population parameters as mentioned earlier) during the early to late interval, the proportion of animals reacting to the test as false positives during the late phase of the program is accentuated. Progress in the MG control program currently seems to be at this latter stage, and may explain why we are observing a greater number of false positive reactors. It is possible that the test is not at fault, but rather the population is now essentially nondiseased so that a high proportion of serologic reactors are actually false positive reactors. The following is a hypothetical problem to bring out the above point:

Assume: (1) Sensitivity of the MG agglutination test is 98%: specificity is 99%.

(2) Population parameters have not changed (i.e., sens-spec. values have remained constant over the years).

(3)

Period of Control Program	MG Prevalence	Number Tested	Diseased	Non-diseased
Early (1950)	20%	10,000	2000	8000
Late (1975)	1%	10,000	100	9900

(4) With data from (1) and (3) and using the four-fold classification scheme, we can calculate the false positive rates as follows:

a) Early:

	+	-	Total
+	1960	80	2040
-	40	7920	7960
	2000	8000	10000

False + = $\frac{80}{2040}$ = 3.9%

b) Late:

	+	-	Total
+	98	99	197
-	2	9801	9803
	100	9900	10000

False + = $\frac{99}{197}$ = 50%

Another important point when testing populations with low disease prevalence is that a small decrease in the specificity of the test will greatly increase the number of false positives. In the previous example (b), if we maintain sensitivity at 98% but change specificity to 98%, the false positive rate will be 67% which amounts to a 17% increase in false positives by reducing the specificity by 1%. This type of decrease in specificity may be due to faulty antigens or improper test procedures, or there may have been a real change in population parameters, e.g., a cross-reacting antigen may have been introduced into the population.

Problems of False Negatives. Assuming no changes in the population and screening test parameters, the false negative rate of a screening test should decrease as the prevalence of the disease decreases (similar mathematical relationship as used for false positives). However, during the late stages of a program one might become more aware of false negatives since the yield of true positives is low (on a population basis). The problem of false negatives probably existed to the same extent during the early stages of the program, but due to the high case load of true positives, little attention may have been given to this problem during early stages of control. Another possible explanation for the current problem of false negatives may be that the host has become more resistant (through natural selection) or there may have been an antigenic shift of the organism.

Summary. I have attempted to place in perspective the current problems we are facing in interpreting MG serology. It is a truism that for the more successful disease control programs where prevalence of disease is reduced significantly, the deficiences of the screening test(s) are accentuated. Under such circumstances, studies as reported by many workers at this symposium to correlate screening test results to true infection/disease status must be continued if we are to minimize the incorrect decisions in our testing programs. Alternate (supplemental) tests and test procedures including the use of variant strains for antigen should be considered. The concept of a "significant titer" when conducting the rapid plate test may be important. Finally, we should recognize that false negatives and false positives may on the one hand be "man-made" via improper handling of sera and antigens, or even improper application and interpretation of the test, and on the other hand, may have biologic origin, inherent in the host, the agent and the environment of their interaction.

Reference:

Thorner, R. M. and Remein, Q. R. 1961. Principles and procedures in the evaluation of screening for disease. Public Health Monograph 67. U.S. Govt. Printing Office.

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July 31, 1975

Dr. Frank J. Siccardi
2850 Inwood Lane
Fayetteville, Arkansas 72701

Dear Frank:

The following comments are intended to reflect my evaluation and conclusions drawn from the reports, and discussions at the Mycoplasma Workshop. Unavoidably, the conclusions are supported by my own personal experience.

1. The workshop did an outstanding job of presenting a well organized perspective of the total problem.
2. Dr. Mallinson presented typical cases of what is seen in the field by many people.
3. Dr. Yoder further supported what Dr. Mallinson and others are experiencing with isolation data. It is well accepted that the reactions are weak, transitory in many cases, and with only a portion of the flock showing a positive serum - plate reaction at any one time. Isolations are difficult, and the virulence of the isolates is of a low order, as observed with air sac condemnations.
4. Unquestionally there are non-specific reactions caused by stimuli other than Mycoplasma. Dr. Olson has some suggested techniques for identifying non-specific reactions.
5. Drs. Kleven and Adler gave support to serological variation among isolates. This has been recognized a long time and is well documented. Dr. Klevin's work shows some deviation. It is reasonable to expect there are isolates that are more or less devious than Dr. Klevin showed. Dr. Yoder very strongly emphasizes that they are MG as far as he is concerned. I find no serious fault with that, except that I have tested some that were closer to MS than MG. The real problem is:"Are they devious enough that special Antigens and procedures are required to adequately detect and differentiate them from the classical MG and MS?"

6. There is definitely a wide gap between the classical problem of MG and MS and the atypical problem. There is a wide difference in both the serology and the clinical manifestations.
7. It is my opinion that these atypical problems are sufficiently devious to warrant a special set of criteria for diagnosis and especially eradication. It appears obvious that the classical problems of MG and MS have been eradicated or nearly so. Remaining is a special problem of low virulence mycoplasma that are sufficiently different serologically to demand the use of special antigens, special testing procedures and special measures for eradication. <u>There may be some questions as to the economic justification for the eradication of all mycoplasma</u>.

As new and old isolates are studied, the virulence, clinical manifestations and serological reactions should be evaluated under both ideal and stresstype environmental conditions. Either may be intensified by stressors. If they are, we need to know it, in order to properly assess the need for eradication of devious isolates.

For your file, I am enclosing copies of two tables. No, 1 was developed by Dr. Kleckner in the early 1950's. I don't know the source of No. 3, other than it was a hand-out at the 1957 collaborators conference on Mycoplasma. To say, the least - they are interesting in light of your workshop efforts.

The committee under your leadership did an outstanding job with a difficult and very controversial subject. The meeting at Davis and the workshop at Anaheim will do much to resolve this agravating problem. You are to be commended for your aggressiveness and leadership in getting the problem before AAAP.

With kindest personal regards, I am

Sincerely,

Frank R. Craig, D.V.M.
Director of Health Services

FRC:mmb
Enclosures

Table I

Serologic Differentiation of Avian PPLO

Agglutination (tube) and Cross Agglutination Tests

Rabbit Antisera Strains	54-537	HPR#5	DIVA	Tu	DPR#2	S-6	ASL	Storrs
54-537 (NP)	80	80	-	-	-	40	-	-
HPR#5 (NP)	80	80	-	-	-	-	-	-
DIVA (P)	(40)	-	640	320	(20)	-	-	-
Tu (NP)	20 80	-	160	320	20	-	-	-
DPR#2 (P)	-	-	-	-	160	-	-	-
S-6 (P)	-	-	80	-	(40)	320	(40)	(40)
ASL (C.A.)	-	-	(-)	-	(-)	40 80	(160)	20
Storrs (C.A.)	-	-	-	-	-	10 20	20	160

- No agglutination at 1-10 dilution after 48 hrs. at 37 C
(40) Not replicated
-20 Range on replication
40 80
(NP) Nonpathogenic strain
(P) Pathogenic strain
(C.A.) Commercial antigen

Table III

Serologic Relationship of Strains of Pleuropneumonia-Like Organisms

ANTIGENS	ANTISERA*				
	T1	C5	C8	Commercial	E. Coli # 1
T1	+ (1:16)	-	-	+ (1:16)	-
C5	-	+ (1:16)	-	+- (Undiluted)	-
C8	+ (1:32)	-	+ (1:64)	+ (1:64)	+ (1:64)
C7	-	-	-	+- (Undiluted)	+- (Undiluted)
C6	+ (1:64)	-	-	+ (1:64)	-
C3	-	-	+ (1:8)	+ (1:4)	-
C2	+ (1:8)	-	+ (1:4)	+ (1:8)	-
ASL	+ (1:4)	-	-	+ (1:32)	-
Conn.	+ (1:4)	-	-	+ (1:8)	-
E. Coli #1	-	(Undiluted)	+ (1:32)	+ (Undiluted)	(1) +(1:64-highest dilution checked)

HI TEST - Using Hofstad No. 801 as Antigen

+ (1:40)	-	-	+ (1:320)	-

*Number in parenthesis is end point dilution of antisera at which agglutination occurred.
(1) Not significant because sera came from chickens that may have had E. Coli infection.