Mycoplasma Committee (1974-75)
Report #1

The Mycoplasma Committee of the AAAP met at 1:30 PM in the Southeast Region Poultry Research Laboratory , Athens, Georgia, September 30, 1974. The following committee members were present. F.J. Siccardi, Chairman. J. Fabricant, R. Yamamota, W. Staples, H. Yoder, S. Kleven and D. Johnson.

Considerable discussion was held in an attempt to pinpoint the major problems in the MG and MS testing and eradication programs. There was agreement that major problems do exist. The problem areas as below, were outlined. It was felt that in order to develope a more full understanding of the various types of problems, that a thorough documentation of selected cases be presentated at a symposium to be held in conjuction with next years AVMA meeting or by appropriate report to the membership, would then be the basis for further research and or action. In addition further meetings and/or wet labs at the California meeting in the winter and North Central meeting in June would be appropriate.

Current problems being encountered in the field with MG and MS Test and Eradication Programs.

A. Flock type situations
   1. Transient serological positive reactions.
      a. The serology changes from negative to positive to negative in a short period of time (30 days or less)
      b. Up to 100% serum plate reactor rate
      c. Both serum plate reaction and HI test results may give typical positive reaction (4+ clumping on serum test, HI titer of 80 etc.)
      d. A lack of disease occurrences in the principle and/or progeny.
      e. A failure to isolate mycoplasmas in reacting birds has been documented.

   2. Low and slow serological results
      a. 30% or less showing slow (atypical-fine) serum plate reaction which may occur after 3 minutes.
      b. HI titers of plate reacting birds may show no to titers or titers of 10, 20 to 40.
      c. type reaction may linger for many months
      d. Culture of flock may be positive and low slow serum plate reaction remain or typical reaction as in 3 below develope.
      e. Flock may eventually test negative (serum plate etc.) and cultural results negative.
      f. Flock continues to test positive (serum plate etc.) at a low rate and culture results remain negative.

   3. Typical positive serological and cultural diagnosis.
      a. Abrupt high % of positive birds in a flock (75%), positive cultural results, serologically positive remainder of life.
      b. Classical disease occurrence in principles and/or progeny.
      c. Atypical disease occurrence (no clinical problems) in principles or progeny.

B. Committee recommendations

1. Thorough documentation of the above 3 type and sub-type flock occurrences is recommended particularly 1 and 2 as below:

a. A complete flock history and clinical working as possible.

1. Parent source and blood test history
2. Blood test history of flock in question
3. Clinical history of flock in question
4. Serological documentation of banded birds (be sure to include antigens used, serial etc.)
5. Culture of reactor 10-20 birds for M. gallisepticum and M. synoviae according to the following recommendations.

CULTURE OF REACTOR BIRDS FOR M. GALLISEPTICUM AND M. SYNOVIAE:

Culture Medium:

Any medium previously established as suitable for the culture of M. gallisepticum or M. synoviae will be satisfactory. An example of a widely used medium which is suitable for the avian mycoplasmas is Frey's medium. A commonly used formulation is attached.

Culture of suspect materials in broth is definitely more sensitive than direct plating on agar. However, a high isolation rate of M. synoviae is usually obtained after streaking of tracheal swabs directly on the agar surface and incubating for 3-4 days.

Selection of Materials for Culture:

A minimum of 10-20 banded reactor birds should be selected for culture from each flock with suspicious serological results. Tracheal swabs or sinus exudates can be cultured at intervals without destroying the birds. At times, repeated cultures or culture of additional birds may be necessary. If reactor birds are to be necropsied, culture of ovary, oviduct, and air sacs, as well as tracheas, is recommended.

Non-pathogenic mycoplasmas frequently contaminate cultures obtained from the upper respiratory system. In this event, inoculation of experimental birds or fluorescent antibody serotyping of colonies on agar may be helpful.

For M. gallisepticum-suspicious breeding flocks, culturing of the yolk sacs of 100-150 dead-in-shell pipped embryos has been shown to be a good method for isolation of the organism.

Shipment of Mycoplasma Cultures:

A. Primary isolation

Tubes containing about 5 ml. of medium should be inoculated and incubated at 37°C overnight. If possible, they should then be frozen on dry ice and shipped to the laboratory by the quickest means available. If dry ice is not available, shipping on wet ice (or even without ice) is generally satisfactory.

B. Established cultures

Established cultures can be shipped as above. It is often helpful to ship 24 hour cultures as well as freshly inoculated cultures. Colonies on agar medium survive shipment nicely--often better than broth cultures. Agar cultures usually survive shipment without refrigeration for several days. Unfortunately, the agar often jars loose from the plate during shipment and arrives as an unusable mass. Perhaps it is best to include both agar and broth cultures.

FMS Medium

For the cultivation of avian mycoplasmas.

Mycoplasma Broth Base (Pfizer)	22.5 g
Dextrose	1.5 g
NAD-Cysteine (1% solution of each)	20 ml
Swine Serum	120 ml
Phenol Red (1%)	2.5 ml
Potassium Penicillin	1 million units
Thallium Acetate (10%)	5.0 ml
Distilled Water	880 ml

Adjust pH to 7.8 with 20% NaOH. Medium may be sterilized by filtration. Alternatively, the water, dextrose and base may be autoclaved. Sterile solutions of the other ingredients may be added aseptically after cooling to approximately 50C. We then dispense in sterile tubes, about 5 m./tube.

For agar plates, a final concentration of 1% is used. We use Inogar #2 (Colab). The agar can be melted by autoclaving. One method is to mix sterile medium made up to double strength and add an equal volume of 2% agar, with both solutions at about 50C. Another way is to sterilize the base, agar, destrose, and water by autoclaving, cool to 50C, and aseptically add the remaining ingredients.

For culture in broth of potentially contaminated specimens, 0.1 ml of 1% Thallium acetate and 0.1 ml of penicillin (10,000 units) may be added to each 5 ml of broth immediately before use.

C. Further committee considerations on problems encountered in Mycoplasma control.

1. Lack of states laboratory capability to culture and isolate Mycoplasma in most states.
   a. In the interim and until the appropriate expertise is identified and/or developed, it is recommended that assistence of one of the following individuals be solicited
      Dr. Kleven, UGA
      Dr. Yoder, USDA, Athens, Ga.
      Dr. Pomeroy, U. of Minn.

Dr. Fabricants, Cornell
Drs. Yamamoto and Adler, Univ. of Calif.
Dr. Hall, Texas A and M
Dr. Vardeman, USDA, State College, Miss.

2. Lack of standardized serological testing techniques in all official testing laboratories.
3. Antigen variability in sensitivity by lots and manufacturers.
4. Recognition of need to further evaluate more recent field isolates for improved sensitivity or detection of possible variants.
5. The need for USDA to continue production of appropriate Mycoplasma reagents (plate and HI antigens, control serums etc)
6. Regulation of controlled MG vaccination, yet recognizing that it may have a place multiage (commercial egg) farms.
7. It is recommended that an individual solicit serums from flock situations particularly Type 1 for development of a standardized laboratory means to eliminate the apparent false positive reaction and/or develope the area.