MYCOPLASMA GALLISEPTICUM INFECTION

SLIDE STUDY SET #11

BY

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Mycoplasmas are the smallest free-living organisms and have complex nutritional requirements. They lack a cell wall, which results in pleomorphism, penicillin resistance, and susceptibility to environmental factors. Thallous acetate and penicillin are added to growth media to inhibit bacterial and fungal contaminants.

Eleven species of avian mycoplasma have been characterized. The original serotyping schemes described 19 serotypes, each designated by a letter (A through S). Subsequently, several serotypes were found to be closely related and grouped into a single species. For example, serotypes I, J, K, N, Q, and R are closely related and have been named Mycoplasma iowae. Serotype A is M. gallisepticum. It is likely that several undescribed species exist.

M. gallisepticum and M. synoviae are pathogenic for chickens and turkeys, whereas M. meleagridis is found only in turkeys. M. gallinarum and M. iowae may have minor pathogenic capabilities.

M. gallisepticum (MG) infection is primarily a respiratory disease, with lesions of tracheitis and airsacculitis. In turkeys, inflammation and swelling of the infraorbital sinus is a common lesion. Sneezing and rales are common signs, but infection may be silent, especially in older birds. Infection of laying hens may result in decreased egg production. Infected birds remain carriers.

Initial histologic lesions are characterized by the presence of surface exudate, edema, fibrin exudation, and heterophilic and lymphocytic cell infiltration. Air-sacs may be as much as 8- to 10-fold thicker than normal. Multiple foci of epithelial cell

hypertrophy, degeneration, and necrosis probably represent sites of attachment and colonization by mycoplasma organisms. The early epithelial cell changes are followed by hyperplasia. With time postinfection, lymphocytes, macrophages and plasma cells diffusely infiltrate the connective tissue. Nodular lymphoid cell foci are more common in older lesions. End-stage lesions consist frequently of scattered lymphoid nodules in the increased fibrous connective tissue.

MG infection in the U.S. has been virtually eradicated from turkey, broiler and leghorn-type breeding stocks. Infection in growing turkeys and broilers is infrequent. The primary focus of infection in the U.S. is multiple-age commercial layer flocks in which the organism is transmitted to MG-free pullets after they have been moved to the infected premises. Also, infection of back-yard flock and fancy breeds is common.

Synergism between MG and other infectious agents is common. Newcastle disease and infectious bronchitis in chickens are commonly involved, either from vaccines or field infection. Influenza virus may be involved in turkeys. Pathogenic strains of Escherichia coli may also complicate the disease picture and lead to pericarditis, perihepatitis, and mortality in broilers.

Egg transmission is a primary mode of dissemination of MG. In addition, the organism spreads readily by direct contact. Transmission by indirect contact is less likely but is nevertheless an important means of transmission. The organism does not survive more than several hours outside the host.

The criteria for diagnosis are signs and lesions (if present), serological tests, and isolation and identification of the organism. Signs and lesions or respiratory infection are not pathognomonic, and laboratory confirmation is essential. Serum samples from suspect flocks are tested by the plate-agglutination test. If positive, the diagnosis can be confirmed by the hemagglutination-inhibition (HI) test. Generally, HI titers of 1:40 are considered suspicious, and 1:80 or higher is considered positive. Because of the tendency of the agglutination test to give occasional false-positives and relative lack of sensitivity of the HI tests, the results must be interpreted with care. Also, HI titers may be delayed 1 week or more after agglutination antibodies are detected. Frequently, retests are necessary.

Several media have been used for the isolation of MG. Perhaps, the most widely used in the U. S. is Frey's medium (Table 1). Generally, inocula are obtained from cotton swabs of respiratory tissues (trachea, sinus, airsac), and tubes of broth medium are inoculated and incubated at 37 C. When the pH drops because of glucose fermentation (color change of phenol red indicator from red to yellow), broth cultures are plated on agar medium and incubated at 37 C. The initial color change of the phenol red indicator may occur from 2 to 14 days postinoculation or longer. Typical mycoplasma colonies may be observed as early as 24 hours postinoculation, but MG colonies are not visible for 3 to 5 days. Agar plates should be examined microscopically (35-100X) under low light for typical colonies.

Because avian species may harbor many mycoplasma serotypes, isolates must be serotyped. The most commonly used methods are growth inhibition with specific antisera, immunodiffusion, and immunofluorescence of mycoplasma colonies. Immunofluorescence has the advantage of quickness and ability to detect MG in mixed cultures, but specific conjugates are not widely available.

Prevention and control centers around the maintenance of MG-free breeding stock. Serological monitoring programs for breeder flocks and replacement pullets is an essential feature of this program. Specific details of such programs are given in the National Poultry Improvement Plan. Antibiotic treatment or heat treatment of hatching eggs from infected flocks has been used to reduce the level of egg transmission. Egg-treatment methods and vaccination of replacement pullets with live MG cultures during the rearing period have been used to eliminate MG infection from valuable genetic stock. Isolation and sanitation methods are used to maintain flocks free of infection.

In some states, the F strain of MG has been given to replacement pullets as a live vaccine during the rearing period. This helps prevent the production drops that commonly occur when uninfected pullets are moved to infected, multiple-age commercial egg farms.

A USDA-licensed, inactivated, oil-emulsion bacterin recently has been introduced. Chickens or turkeys vaccinated subcutaneously or intramuscularly have been shown to be protected against clinical signs and lesions associated with MG. The primary use of this

product will be to reduce egg-production losses on MG-infected, multiple-age commercial egg farms. The bacterin has also been shown to reduce the level of egg transmission.

Treatment of MG-infected flocks is an aid in preventing respiratory signs, airsacculitis, and drops in egg production, but results are not always satisfactory. Tylosin, tetracyclines, erythromycin, a combination of spectinomycin and lincomycin, gentamicin, and other broad-spectrum antibiotics have been used. Antibiotic treatment is not satisfactory for the elimination of the carrier status.

TABLE 1. MYCOPLASMA BROTH MEDIUM (FREY)

	Per Liter
Deionized distilled water	880.0 ml
Thallous acetate (10% sol.)	5.0 ml
Penicillin (aqueous)	500,000 units
Mycoplasma broth base*	22.5 g
Seine serum (heated 56 C for 30 min)	120.0 ml
Dextrose	3.0 g
Phenol red (1% sol.)	2.5 ml
NAD (1% sol.)	12.5 ml
Cysteine hydrochloride (1% sol.)	12.5 ml

Adjust to pH 7.8 Filter Sterilize

Add thallous acetate to the water first to avoid precipitation of proteins of media and serum. Horse serum is adequate for MG, but swine serum is best for M. synoviae. Cysteine hydrochloride is added to reduce the NAD (beta nicotinamide adenine

dinucleotide) which is required for the growth of M. synoviae. For agar plates 1.5% agar is used.

*Mycoplasma broth base (frey)

Product # M 33600	Product # 3900 - 3212
Gibco Diagnostics	Scott laboratories, Inc
2801 Industrial Drive	8 Westchester Plaza
Madison, Wisc. 53711	Elmsford, N.Y. 10523

NAD Grade III	Thallous acetate
Sigma Chemical Co	Fisher Scientific Co.
P O Box 14508	2775 Pacific Drive
St. Louis, MO 63178	P O Box 829
	Norcross, GA 30091

SLIDE 1. Eleven species of avian mycoplasma have been characterized. The original serotyping schemes described 19 serotypes, each designated by a letter (A through S). Subsequently, several serotypes were found to be closely related and grouped into a single species. For example, serotypes I, J, K, N, Q, and R are closely related and have been named Mycoplasma iowae. Serotype A corresponds to MG. It is likely that several undescribed species exist.

SLIDE 2. Swollen sinuses are a common sign of MG infection in turkeys but not chickens. Although there are other causes of sinusitis, MG infection must be eliminated as a cause whenever swollen sinuses are observed in a turkey flock.

SLIDE 3. Normal air sacs of a chicken. They should appear as a thin, transparent membrane.

SLIDE 4. Air-sac lesion of the posterior thoracic air sac of a broiler chicken. There are many causes of air-sac lesions of chickens and turkeys, but MG should always be considered in the differential diagnosis. Normal air sacs should appear as a thin, transparent membrane.

SLIDE 5. Acute MG airsacculitis of the abdominal air sac of a broiler chicken. This lesion would result in condemnation of the carcass if observed at the processing plant.

SLIDE 6. Pericarditis and perihepatitis are common sequelae when Escherichia coli infection occurs in MG-infected chickens. Mortality, uncommon in uncomplicated mycoplasma infections, may be severe when E. coli infection complicates the disease picture.

SLIDE 7. This is a normal air sac showing the simple squamous lining epithelium, scant amount of connective tissue, and outer mesothelial cell layer.

SLIDE 8. This mycoplasma-infected air sac is greatly thickened due to epithelial cell hyperplasia, increased number of blood vessels, and infiltration by heterophils and lymphocytes. Surface exudate is present also. This is a relatively acute lesion (4 days postinfection).

SLIDE 9. Epithelial cells are swollen and the air sac is infiltrated by plasma cells in this example of chronic mycoplasma-induced airsacculitis. Note the mononuclear cells in blood vessels.

SLIDE 10. Lymphoid nodules (lymphofollicular reaction) are prominent in this air sac. Increased air-sac thickness is due also to a diffuse infiltration of lymphocytes, macrophages, and plasma cells.

SLIDE 11. Tracheal swabs can be obtained for mycoplasma culture from live birds. Isolation and identification of the organism is often used in addition to serological tests for diagnosis.

SLIDE 12. The tube of mycoplasma medium on the right is uninoculated. The tube on the left shows a yellow color change of the phenol red indicator because of acid production from glucose metabolism. Cultures should be plated on agar medium when the color change is evident. Nonglucose-fermenting mycoplasma species cannot be detected by the color-change method.

SLIDE 13. Typical mycoplasma colonies as observed by microscopic examination of agar medium at 35X. M. gallisepticum colonies can generally be observed after 3 to 5 days of incubation at 37 C. Since colony morphology of various mycoplasma species is similar, further identification is necessary. The background color of this slide is due to the microscope filter.

SLIDE 14. Immunofluorescence of mycoplasma colonies (35X) in a mixed culture. M. gallisepticum colonies, stained by specific, fluorescein-labeled antiserum, are bright green. Other colonies are less distinct in appearance, and may be barely visible.

SLIDE 15. Identification of a mycoplasma isolate by growth inhibition. Specific antiserum inhibits growth of MG around a serum-impregnated filter-paper disc. No inhibition is observed with antisera against other mycoplasma species.

SLIDE 16. Serotyping by immunodiffusion. Antigen prepared from unknown mycoplasma isolate forms a precipitin line with specific MG antiserum. A line of identity is formed with a control MG antigen. MG_C = Chicken antiserum against MG; MG_R = Rabbit Antiserum against MG; N_R = Normal rabbit serum; N_C = Normal chicken serum; MS_C = Chicken antiserum against M. synoviae; MS_R = Rabbit antiserum against M. synoviae. The center well contains unknown antigen. (Slide courtesy of Dr. H. W. Yoder).

SLIDE 17. Serum-plate-agglutination test with MG antigen. The serum sample on the right is positive, and the sample on the left is negative.

SLIDE 18. Titration of MG hemagglutination antigen. Antigen is diluted twofold from top to bottom beginning with a 1:10 or 1:15 dilution. The titer in this example is 1:320 for 1 unit of HA antigen.

SLIDE 19. Example of HI tests with 3 negative and 3 positive sera. Sera are diluted from top to bottom, beginning at 1:10. Rows 1 and 12 are empty. Sera in rows 2, 3, and 4 are from negative control birds (the top well is a serum control). Sera in rows 5, 6, 7 are from MG-infected chickens. The titers are 1;640, 1:320, and 1:80. Rows 8 and 9 are antigen controls and rows 10 and 11 are cell controls.

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