MYCOPLASMA SYNOVIAE INFECTION

SLIDE STUDY SET #12

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INTRODUCTION

Mycoplasma synoviae (MS) infection was originally designated as infectious synovitis, an acute to chronic infectious disease of chickens and turkeys involving primarily the synovial membranes of joints and tendon sheaths and producing an exudative synovitis, tenosynovitis, or bursitis. However, respiratory infections often including airsacculitis have become the most frequent form of MS infection.

HISTORY, INCIDENCE AND DISTRIBUTION

Infectious synovitis was first apparent during the early 1950's. It soon became almost worldwide in distribution and produced clinical disease in growing chickens 4 to 12 weeks of age. Replacement flocks of egg-laying chickens were sometimes involved. It usually appeared in turkey flocks 10 to 20 weeks of age, but less frequently than in chickens.

During the 1970's, clinical evidence of MS infection as a respiratory disease of chickens, and sometimes turkeys, became more frequent. The number of infectious synovitis cases rapidly declined. By 1980, serological evidence of MS infection was very common, while obvious respiratory infections were increasing and clinical synovitis was relatively rare in both chickens and turkeys.

ETIOLOGY

Mycoplasma synoviae is the classical cause of infectious synovitis of chickens and turkeys. MS more frequently produces a lingering infection of the trachea and sometimes is involved with airsacculitis under certain stress conditions, generally including virus infections.

M. synoviae is a very fastidious bacterium requiring a protein-rich medium enriched with 10-15% swine serum and specifically requires the addition of nicotinamide adenine dinucleotide (NAD).

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
Swine 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 M.

gallisepticum, 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.

*Mycoplasma broth base (Frey)

Product M 33600
Gibco Diagnostics
2801 Industrial Drive
Madison, WI 53711

Product 3900 - 3212
Scott Laboratories, Inc
8 Westchester Plaza
Elmsford, NY 10523

NAD Grade III
Sigma Chemical Co.
P O Box 14508
St. Louis, MO 63178

Thallous acetate
Fisher Scientific Co.
2775 Pacific Drive
P O Box 829
Norcross, GA 30091

COLONY MORPHOLOGY

The colonies on solid media are best observed with a dissecting microscope at 30X magnification using indirect lighting. They appear as raised, round, slightly latticed colonies with or without centers. The colonies are from 0.1 to 0.5 mm in diameter, depending on the number of colonies present, suitability of media, and age of culture.

MORPHOLOGY AND STAINING

In stained preparations MS appears as pleomorphic coccoid bodies or rods approximately 0.2 um in diameter to 0.4 um in length. Staining is accomplished by spreading a thin film of broth on a clean slide and allowing it to dry at room temperature. The film is fixed by flooding the slide with Bouin's fixative for 5 to 10 minutes. The slide is washed in running water until the yellow color in the fixative disappears. Ten drops of Giemsa blood stain is placed in 10 ml of water. The slide is flooded with the diluted Giemsa and allowed to stain for 30 minutes. The stain is washed off with running water and allowed to air dry before observation at 1,0000X magnification.

BIOCHEMICAL PROPERTIES

M. synoviae ferments glucose and maltose with the production of acid, but not gas, in suitably enriched media. It does not ferment lactose, dulcitol, salicin, or trehalose. Some isolates of MS are capable of hemagglutinating chicken and turkey erythrocytes.

RESISTANCE TO CHEMICAL AND PHYSICAL AGENTS

Resistance to disinfectants has not been determined, but MS is probably sensitive to most disinfectants as are other mycoplasmas. M. synoviae is not stable at pH 6.9 or lower. No critical studies have been made on the resistance of MS to heat, but it is believed to be sensitive to temperatures above 39 C. It will withstand freezing, but the titer is reduced. Broth cultures usually survive

storage for at least 10 years at -60 C.

TRANSMISSION

Direct contact of susceptible birds with infected carrier chickens or turkeys causes outbreaks of the disease, or at least tracheal infection. It is also spread by airborne dust or aerosol droplets. Spread by contact with contaminated equipment is generally assumed, but has not been well documented.

Egg transmission of MS is extremely important. Control procedures are founded primarily on methods to prevent egg transmission.

SIGNS

Chickens. The first observable signs of typical infectious synovitis are pale comb, lameness, and retarded growth. As the disease progresses, the feathers become ruffled and the comb shrinks. In some cases the comb is bluish-red. Swellings usually occur around the joints, and breast blisters are common. The hock joints and foot pads are principally involved, but in some birds all joints become affected. However, birds are occasionally found that have a generalized infection by no apparent swelling of the joints. The birds become listless, dehydrated, and emaciated. Although birds are severely affected, many of them continue to eat and drink if placed near feed and water. A greenish discoloration of the droppings, which contain large amounts of uric acid or urates, is frequently seen.

The acute signs described above are followed by slow recovery, but synovitis may persist for as long as 5 years. In other instances, the acute phase is absent or not noticed, and only a few chronically infected birds are seen in a flock. Chickens infected via the respiratory tract may show slight rales in 2 to 4 weeks or show no symptoms, having only serological evidence of infection. However, moderate to severe airsacculitis may occur in conjunction with live virus vaccine or field virus infection during cold environmental conditions.

Turkeys. Infectious synovitis generally causes the same type of signs in turkeys as in chickens. Lameness is the most prominent symptom. Warm fluctuating swellings of one or more joints of lame birds are usually found. Occasionally there is an enlargement of the sternal bursa. Severely affected birds lose weight, but many birds less severely affected make satisfactory weight gains when separated from the flock. Respiratory infections involve primarily the trachea, with resulting serological reactions. However, obvious airsacculitis does occur in some instances as does sinusitis in very rare instances.

GROSS LESIONS

Chickens. In the early stages of infectious synovitis, necropsy reveals a viscous, creamy to gray exudate involving the synovial membranes of the joints, keel bursae, and tendovaginal sheaths. As the disease progresses, this exudate becomes caseous. Caseous exudate is occasionally found over the skull, along the

neck, and rarely extends into the muscles and air sacs. When birds become severely emaciated and dehydrated before caseous exudate develops, there is occasionally no fluid about the joints. In chronic cases the surfaces of the affected joints are frequently yellow to orange.

In the early stages of the disease, splenomegaly generally occurs. The liver is frequently enlarged and occasionally mottled, greenish, or dark red. The kidneys are usually swollen, mottled, and/or pale. These changes occur in approximately 50% of the birds and become more pronounced and frequent as the severity of the disease increases. Even though some birds are severely affected,m their internal organs appear normal. In experimental foot-pad-inoculated birds, the infection frequently localizes in the inoculated foot, and no gross internal lesions are noted.

Although respiratory involvement was rarely significant with clinical infectious synovitis, tracheitis and airsacculitis have become far more common during recent years. Airsacculitis in both chickens and turkeys is usually associated with live virus vaccines or field infections with Newcastle disease in chickens and turkeys, or especially infectious bronchitis in chickens. This is especially true during cold stress periods of the winter months.

HISTOPATHOLOGY

Microscopic lesions observed with typical infectious synovitis were severe and extensive. The brain frequently exhibits vascular endothelial thickening and adventitial proliferation in the

cerebrum, cerebellum, optic lobe, degeneration of some of the Purkinje cells, and occasionally cerebellar lesions similar to those of encephalomalacia.

In the liver, perivascular, periportal, and interparenchymal cellular hyperplasia of the reticular cells of the reticuloendothelial system occur. The sinusoids are dilated and the parenchymal cells are atrophied. There is proliferation of the bile duct epithelium. The connective tissue framework of the heart, gizzard, and interlobular septa of the lungs reveals a similar reticular cell hyperplasia. Occasionally focal mononuclear infiltration and necrosis of the myocardium and a fibrinous inflammation of the pericardium are seen. A reticular cell or lymphocytic hyperplasia, or both, decrease the sinusoidal areas of the spleen. A granulocytic hyperplasia of the bone marrow occurs, and atrophy of the thymus and bursae of Fabricius results from lymphoid degeneration in the medulla and cortex.

MS infection causes tracheitis characterized by edema, then cellular infiltration consisting primarily of lymphocytes, plasma cells, and macrophages. Similarly, airsacculitis is noted by cellular infiltration and hyperplasia of the lining epithelial cells with focal to diffuse areas of desquamation and variable amounts of cellular debris within the air sacs.

DIAGNOSIS

A positive diagnosis of MS infection may best be made by isolation and identification of the infectious organisms. However,

serological tests are frequently relied upon.

SEROLOGY

Antigen is available commercially for the rapid serum plate test. Adequate directions for use are given with each package. Generally, 0.02 ml of the serum is mixed with an equal amount of antigen on a glass plate. The plate is gently rotated and observed against a white background with indirect lighting. Agglutination with most antigens occurs with 2-3 minutes at room temperature. Confirmation of plate reactor serum samples is generally conducted via the hemagglutination-inhibition test. The agar gel precipitin (AGP) test may also aid in confirmation of reactor sera, but not all serum samples from known infected chickens give reactions by the AGP tests. The AGP test is especially useful in typing mycoplasma cultures.

DIFFERENTIAL DIAGNOSIS

Cultural and serological procedures must be employed to differentiate MS infections from viral arthritis, Staphylococcus infection, and especially MG in respiratory infections. Escherichia coli is rarely involved with MG respiratory infections, although it is very common in complicated MG infections.

TREATMENT

A summary of data obtained from field and experimental studies indicates that tetracycline antibiotics, 50 to 100 g per ton of

feed, given continuously, will provide satisfactory control of infectious synovitis. Higher concentrations (approximately 200 g per ton of feed) are needed to control synovitis after infection has occurred in a flock. Severely infected birds show improvement after treatment, but such a procedure is not considered practical.

M. synoviae airsacculitis is broilers sometimes responds to several days of feed treatment with tylosin or one of the tetracycline antibiotics, but use of antibiotics are probably more effective in the prevention of airsacculitis. Two grams of an antibiotic mixture composed of 1 part lincomycin and 2 parts spectinomycin per gallon of drinking water given for the first 5 days of age has been found to greatly reduce MS airsacculitis in broilers.

PREVENTION AND CONTROL

Since MS may be spread by direct or indirect contact, in addition to egg transmission, the propagation of progeny from known clean flocks within isolation facilities is essential. MS-free breeding chickens are readily available due to extensive voluntary control programs based on repeated serological testing of flocks.

Monthly testing of only 10 to 100 serum samples by the rapid serum-plate test is generally adequate to detect infected flocks. Positive plate reactions are confirmed by the hemagglutination-inhibition (HI) test. Some false-positive plate reactions may occur at times. They are usually found to be negative by the HI and AGP tests and are only transient reactions.

REFERENCES

1. Frey, M.L., R.P. Hanson, and D.P. Anderson. A medium for the isolation of avian mycoplasmas. Am. J. Vet. Res. 29:2163-2171. 1968

2. Hamdy, A.H., S.H. Kleven, E.L. McCune, B.S. Pomeroy, and A.C. Peterson. Efficacy of Linco-Spectrin water medication on Mycoplasma synoviae airsacculitis in broilers. Avian Dis. 20:118-125. 1976.

3. King, D.D., S.H. Kleven, D.M. Wenger, and D.P. Anderson. Field studies with Mycoplasma synoviae. Avian Dis. 17:722-726. 1973.

4. Kleven, S.H., O.J. Fletcher, and R.B. Davis. Influence of strain of Mycoplasma synoviae and route of infection on development of synovitis or airsacculitis in broilers. Avian Dis. 19:126-135. 1975.

5. Kleven, S.Y., D.D. King, and D.P. Anderson. Airsacculitis in broilers from Mycoplasma synoviae: effect on air-sac lesions of vaccinating with infectious bronchitis and Newcastle virus. Avian Dis. 16:915-924. 1972.

6. Nonomura, I., and H.W. Yoder, Jr. Identification of avian mycoplasma isolates by the agar-gel precipitin test. Avian Dis. 21:370-381. 1977.

7. Olson, N.O. Mycoplasma synoviae infection. In: Diseases of Poultry, 7th ed. M.S. Hofstad, B.W. Calnek, C.F. Helmbolt, W.M. Reid, and H.W. Yoder, Jr. eds. Iowa State University Press, Ames, Iowa. 233-270. 1978.

8. Sahu, S.P. and N.O. Olson. Evaluation of broiler breeder flocks for nonspecific mycoplasma synoviae reaction. Avian Dis. 20:49-64. 1976.

DESCRIPTION OF SLIDES

1. Slide showing typical MS colonies (30 X) on agar medium following 3-5 days moist incubation at 37 C. The colonies are smooth, with entire borders and generally contain a dense elevated center. Colonies are rarely greater than 0.10 to 0.30 mm in diameter. It is not possible to distinguish serotypes by their colony formation, although many nonpathogenic serotypes tend to have colonies up to 1.0 mm.

2. One method of keeping mycoplasma-inoculated agar plates in a moist environment during 37 C incubation for several days is to enclose the plates in a shallow pan containing a water-moistened gauze pad. Aluminum foil or plastic film readily seals the pan. Some workers prefer a sealed candle jar. This assures moisture, and there may be some benefits afforded by increased CO_2.

3. Tube of liquid mycoplasma growth medium showing an oily pellicle or film on the surface. This is especially characteristic of initial MS growth after several days of incubation. The yellow color of phenol red in the medium indicates an acid pH due to MS fermentation of dextrose.

4. Mycoplasma cultures isolated from trachea swabs or air-sac lesions must be serotyped. M. synoviae and M. gallisepticum are only 2 of some 20 possible serotypes of avian mycoplasma. This slide shows the greenish glow of colonies that are positive in the fluorescent-antibody (FA) test.

5. A somewhat more simple method of typing cultures is available by preparing antigens from culture isolated for use in the AGP test. This slide shows the typical precipitin reaction seen when MS antigen is placed in the center well of the agar and test sera are placed in the surrounding wells. Nc = normal chicken serum; MSc = serum from known MS-positive chicken; MSr = serum from MS-inoculated rabbit; Nr = normal rabbit serum; MGr = serum from MG-inoculated rabbit; MGc = serum from known MG-positive chicken.

6. Chicken with its tongue pulled aside to position the larynx for insertion of a cotton swab to obtain tracheal exudate for cultivation of most avian mycoplasma, including MS. Tracheal infection tens to persist for several weeks to months.

7. Normal air-sac membranes are so thin and clear that they are almost invisible. The air sacs are primarily paired extensions (thoracic, abdominal, etc.) of the air passages from the bronchioles on out beyond the lungs into various body cavity spaces. Some are within hollow bones. This slide shows a moderately inflamed air sac as noted by slight thickening of the membrane, some cloudy exudate, and increasing flecks of yellowish caseous exudate as the process continues.

8. Extensive airsacculitis denoted by severe thickening of the air sac membrane with large masses of caseous exudate containing cellular debris and increased vascular activity as the lesion is starting to regress. It is rarely possible to isolate mycoplasma organisms from such chronic lesions.

9. Nearly normal chicken air-sac section showing delicate structure, although there is slight edema, since this chicken received IBV 5 days before. 480 X. (Slide courtesy of Dr. W. T. Springer).

10. Greatly thickened air sac, infected 17 days previously with MS and 12 days previously with IBV; degenerating heterophils are present, and mononuclear cell infiltration and vascularization has occurred. 480 X. (slide courtesy of W. T. Springer).

11. Mesobronchus, infected 10 days previously with MS and 5 days previously with IBV. Extensive mononuclear cell infiltration and lymphoid follicle formation in the submucosa. 480 X. (Slide courtesy f Dr. W. T. Springer).

12. Enlarged foot pads are typical of infectious synovitis produced by MS. Upon incision, a viscous watery fluid almost flows out, but becomes more yellowish and caseated as the lesion becomes older and more chronic.

13. Broiler chicken with both hock tendon areas swollen from MS-induced infectious synovitis. The swelling is produced by inflammation of the synovial lining of the joint capsule and/or tendon sheaths.

14. Incised swollen hock joint of chicken with MS synovitis. Thickened, yellowing fluid is rather clear but becomes more caseous as the duration of infection lengthens or if other bacterial contamination occurs. MS may be isolated from early lesions, but toxic effects from too large an inoculum should be avoided by making a culture transfer in 24 hours. (Slide courtesy of Dr. N.

O. Olson).

15. Cross-section of tarsometatarsal tendon of chicken infected with MS 10 days previously. Extensive accumulation of heterophilic exudate in tendon sheaths and between tendons. 25 X. (Slide courtesy of Dr. K. M. Kerr).

16. The rapid serum-plate test is a very useful serological test for the presence of antibodies for MS in the blood serum of infected chickens or turkeys. Agglutination of the rose-bengal-stained antigen is noted by aggregates or clumps of MS organism by specific antibodies produced by the infected birds.

17. The hemagglutination-inhibition (HI) test is employed to confirm most preliminary rapid serum-plate or agglutination reactions. The HI system uses normal chicken (or turkey) red blood cells to detect the presence of very specific antibodies that inhibit agglutination of the red blood cells by the organism in the antigen. This slide shows a typical micro-test plate employed in the HI test for MS. From left to right, there are 11 rows of serum titrations, and the 12th row is a hemagglutination (HA) antigen titration. There are 8 dilutions of each serum, beginning with 1:10 in the top well doubling to 1:20; 40, 80, 160, 320, 640 and 1:1280 in the 8th (bottom) well. Solid pink-red sheets of agglutinated chicken red blood cells (0.5% in phosphate-buffered saline) indicate complete HA by the antigen Smooth red dots in the center of the bottom of the wells indicates complete inhibition of the red-blood-cell (RBC) agglutination. Thus, the HI titers are recorded as the highest dilution of serum that completely inhibits

RBC agglutination. The first 8 rows contain sera from an MS-positive flock, although samples 2 and 5 are essentially HI-negative. Row 9 contains a negative control serum with a typical reaction of 1:10 (or less). Row 10 indicates a weak reactor serum with an HI of 1:80. The strong positive control serum in row 10 has a titer of at least 1:640; the reaction at 1:1280 is partial. Row 12 contains a re-check on the antigen titration, starting with undiluted antigen as prepared to contain approximately 4 HA units. The second well contains a 1:2 dilution, doubling on through 1:4, 1:8, etc. The HA activity appears to be complete at 1:4 but negative at 1:8. This confirms that the antigen used in the test contained a little less than 4 HA units per well. (There should be partial HA activity at the 1:8 dilution if a full 4 HA units are present in the undiluted preparation). When using 4 HA units of antigen in each well, normal chicken sera will generally not react or will react only at 1:10 or possibly at 1:20. Positive HI tests are considered to be represented by serum titers of 1:80 or greater. Reactions not greater that 1:40 are considered "suspicious" and suggest that HI tests be conducted on sera from that flock 10-14 days later. Continued reactions of questionable titers usually indicate the need for 10 to 20 tracheal cultures sometimes repeated at 2-week intervals to facilitate determination of the flock status.

18. Schematic representation of the mechanism of egg transmission of MS (and M. gallisepticum). Infected chicken with involved trachea and air sacs which sometimes contaminate the

adjacent infundibulum of the oviduct, causing normal ova to become M. synoviae-infected during their passage through the first part of the oviduct. The first evidence of mycoplasmal infection appears within the yolk-sac membrane of developing embryos. Egg transmission may never occur at a rate beyond 2-10% within a given flock, and it becomes less with time, but it is a very important means of transmission and must be eliminated to provide complete control of the infection.

19. The pre-incubation heat treatment of hatching eggs has been employed to eliminate egg transmission of MG and MS from infected flocks to their progeny. The eggs are heated to an internal temperature of at least 46 C during a 12-to-14 hour incubation period. This inactivates the mycoplasma while generally reducing hatchability not more that 3 to 5%.

20. Area of swollen tendons, especially above the left hock, of a chicken with tenosynovitis caused by viral arthritis infection. It is essential to differentiate these two diseases, by serology, cultivation of the causative agent, and/or histopathology. (Slide courtesy of Dr. N. O. Olson).