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DEVELOPMENTS IN VETERINARY SCIENCE

MYCOPLASMA INFECTIONS WITH EMPHASIS ON THE CONTROL OF *M. GALLISEPTICUM*

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Mycoplasma is the species classification given to those organisms commonly known as PPLO or pleuropneumonia-like organisms. Bovine pleuropneumonia was well known in Europe and Asia for many years prior to 1898 when Nocard and Roux discovered a filterable organism that could also produce visible bodies similar in some respects to the bacteria. This organism is now called *Mycoplasma mycoides* (pleuropneumonia organism–PPO). Some of the characteristics of the *Mycoplasma spp.* are given in Table I. Important points to note are that: they are the smallest free-living organisms, in some cases smaller than the large viruses; they can live and reproduce on synthetic media; they have no rigid cell wall and are resistant to penicillin.

Several important points on the pathogenicity of Mycoplasma are given in Table II. It should be pointed out that there are many non-pathogenic mycoplasma, one example is *Mycoplasma laidlawi* which is frequently found in sewage. Mycoplasma are very fragile organisms and as a result, it is believed that transmission is generally by direct contact from one bird to another or from one animal to another. The organism is rather slow growing and this could account for a slow rate of transmission and a slow rate of spread throughout the flock or herd. Generally, the mycoplasma are not rapid acting pathogens and frequently have to be assisted in their pathogenic activity by other organisms such as viruses, *Escherichia coli* or other bacteria. As a rule, onset of the mycoplasma-caused disease condition is slow and the condition is generally chronic. The animals or birds are sick for protracted periods of time. Some investigators believe that there seldom is complete recovery, at least in a time factor of months. Such appears to be true with *M. gallisepticum*, the chronic respiratory disease (CRD) organism in the avian species. Possibly this phenomenon may be due to the fact that the mycoplasma do live and reproduce within the cells of the host and so may be protected from antibodies or other body defence mechanisms. However, antibodies are produced and these can be detected by agglutination tests, complement-fixation tests and precipitation tests. Some mycoplasmas such as *M. gallisepticum* will cause hemagglutination and so the hemagglutination-inhibition test can also be employed. These tests are generally read on a flock or herd basis. If even a small percentage of the birds or animals in the flock or herd are positive, the whole group is considered positive.

Vaccines have been utilized in an attempt to stimulate immunity. The most commonly used mycoplasma vaccines are made with *M. mycoides*, the bovine pleuropneumonia organism, prepared from fresh tissue extracts or grown in avian embryos or tissue culture. However, these have to be used as living vaccines and are considered to be variable in effectiveness.

Mycoplasma are difficult to grow especially on initial isolation and this makes diagnostic confirmation frequently difficult. Mycoplasmas are believed to cause damage in the connective tissues attacking the lungs, air sacs, joints, serous membranes and even the arteries of the brain. Generally, a specific mycoplasma will cause disease in only one species of animal or bird and not another. There may be some exceptions to this rule as there are indications that the *M. hominis* type 1 of man is sero-

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TABLE I

ORDER: Mycoplasmatales
FAMILY: Mycoplasmataceae
GENUS: Mycoplasma (PPO., PPLO.)

1. The smallest free-living organisms (0.1 microns (1000 Å) — .25 microns (2500 Å). Midway in size between the viruses and the small bacteria. (Compare with hydrogen atom OE 1 Å.)
2. Can live and reproduce on synthetic media. Needs a complex medium, however, generally containing serum and yeast autolysate.
3. No rigid cell wall (similar to protoplasts). No mucopeptide polymers associated with bacterial cell wall.
4. Resistant to penicillin (due to the fact that there is no cell wall).
5. Replicate by means of an "elementary body" and binary fission.
6. Pleomorphic (due to no cell wall present).
7. Intracellular and extracellular.
8. A sterol or similar substance needed.
9. D.N.A. with low guanine-cytosine ratio.

TABLE II

MYCOPLASMA PATHOGENICITY

1. Many non-pathogens – saprophytes (*M. laidlawei*).
2. Fragile – therefore, transmission generally *direct.*
3. Slow growing – therefore, transmission rate slow and so slow spread through flock or herd.
4. Generally *not* an acute pathogen – often assisted by other organisms, e.g. *E. coli*, viruses, etc.
5. Slow onset generally and *chronic* – sick for long time.
6. Seldom complete recovery (intracellular organism protected?).
7. Antibodies produced – partial recovery (but organism remains?).
   —agglutination, complement fixation
   —with some e.g. *M. gallisepticum* – H.I.
   —results judged on a flock basis.
8. Vaccines – generally live and not highly effective.
9. Difficult to grow on initial isolation – makes diagnostic confirmation difficult.
10. Affects connective tissues of lung, air sacs, joints, serous membranes, arteries of brain.
11. Generally species specific (*M. hominis* I of man = M. arthritidis of rats?)
    (*M. gallisepticum* affects general avian species.).

logically similar to *M. arthritidis* of rats. Also *M. gallisepticum* affects several species but these are generally avian including chickens, turkeys, pheasants and quail.

Examples of the pathogenic mycoplasma are given in Table III. This gives an indication of the many different species of animals that are attacked by the pathogenic mycoplasma. In the bovine species, the type of organism is *M. mycoides*, which causes bovine contagious pleuropneumonia and involves the lungs and pleura. Recently a purulent interstitial form of bovine mastitis has been attributed to infection with mycoplasma. Sheep and goats are also involved with a *M. mycoides var. caprine* but this variety appears to be fairly specific for sheep and goats. Swine have been investigated in the past few years and quite a number of different mycoplasma have been isolated from them including an organism which causes involvement in the lungs, similar to the clinical condition commonly called "enzootic pneumonia of swine". Rats and mice have their own problems with mycoplasma as does the dog and man. The finding that *M. pneumonia* caused "atypical pneumonia" in man has given a tremendous impetus to mycoplasma research in humans which will also benefit the livestock and poultry industry. The avian species have had at least 32 different mycoplasma isolated from them, however, only three are now known to be pathogenic: *M. synovia, M. meleagridis* and *M. gallisepticum.*

M. synovia involves the joints, most commonly in chickens, but can affect turkeys. It was discovered by Dr. Norman Olson and his colleagues at the University of West Virginia and reported in 1954 (13). One of the first reports to the poultry industry was given by Dr. Glen Snoeyenbos in 1954 (15). The first

TABLE III

EXAMPLES OF PATHOGENIC MYCOPLASMA

Species Affected	Name	Disease	Organs Affected
Bovine	M. mycoides *var* bovis	bovine contagious pleuropneumonia	lung, pleura
	M. agalactia *var* bovis	mastitis endometritis	mammary gland uterus & oviduct
	Mycoplasma spp. (unidentified)	polyarthritis pericarditis	joints heart
	M. bovigenitalium	abortion – mastitis	genitalis – udder
Sheep and goats	M. agalactia	contagious agalactia	mammary glands
Goats	M. mycoides *var* capri	caprine contagious pleuropneumonia	lung, pleura
	Mycoplasma spp. (unidentified)	edema disease of Spartan goats	edema of head, limbs – generalized
	Mycoplasma spp. (unidentified)	septicemia, arthritis mastitis (California)	generalized, joints, udder
Swine	M. hyorhinis	polyserositis	serous surfaces
	M. hyopneumonia	chronic pneumonia	lung
	M. granularum	arthritis	joints
	M. hyoarthrinosa	arthritis	joints
	M. hyogenitalium	metritis – mastitis	genitals – udder
Mice & Rats	M. neurolyticum	rolling disease	brain, lung
Rat	M. pulmonis	pneumonia	lung
	M. arthritidis (hominis II)	septic arthritis	joints
Chickens, turkeys and other avian sp.	M. gallisepticum	chronic respiratory disease infectious sinusitis	air sac, lung, trachea, brain sinus, lung, air sac
	M. synoviae	synovitis	joints
Turkeys	M. meleagridis	poult airsacculitis	air sac
Man	M. pneumonia	"atypical" pneumonia	lung
	M. hominis I & II	non-gonococcal urethritis	genital mucous membrane
	M. salivarium	?	mouth
	M. fermentans	?	genital mucous membrane
	M. pharyngis (orale I)	?	pharynx & mouth
	M. orale (orale II)	?	oropharynx
Dog	M. spumens	?	genitals
	M. canis	arthritis	joints
	M. maculosum	urethritis	genitals

case of infectious synovitis in Alberta was suspected, but not proven, in 1955 at the Alberta Veterinary Laboratory (4). In this condition, the synovial membranes in any part of the body can be affected, filling up with a thick gelatinous fluid in response to an inflammation of the joint synovial membrane. Commonly, the synovial membranes of the hock, feet, breast and mandibles are involved. The morbidity varies greatly, but generally is between five and ten per cent; mortality is low, however, birds affected with infectious synovitis are frequently culled as being unprofitable or are killed by their pen mates.

M. meleagridis is believed to be the cause of turkey poult airsacculitis (19). Nearly all

turkey flocks in Canada, U.S.A. and other countries contain birds carrying this organism (16). It reveals itself by producing an airsacculitis in a high percentage of turkey poults both within the egg and at hatch time. It is believed by workers in Western Canada that the turkey poult airsacculitis in some flocks results in leg weakness, broken wing feathers and a deficiency syndrome between three to eight weeks of age (2, 3). The organism is transmitted in the egg and by contact from one bird to another. The latter appears to be either direct, in the case of poults, or by venereal transmission at breeding time (18, 19). Turkey breeders in Canada and the United States are now realizing that this organism is a pathogen and can be causing them economic loss. Control at the present time consists of endeavouring to establish turkey flocks free of *M. meleagridis* by breeding only birds negative on serology or culture and the control of the organism in the egg by dipping fertile eggs in non-toxic antibiotics. Serum plate and tube agglutination tests have been developed for this organism. The antigen is very difficult to grow and standardize. However, this is being attempted on a commercial basis in the United States. At the present time, only laboratories working with *M. meleagridis* have an antigen available and this is in very limited amounts.

M. gallisepticum is believed to be the cause of infectious sinusitis in turkeys and a chronic respiratory disease in chickens. In 1936 Nelson (12) found cocco-bacilliform bodies in a mild, chronic respiratory disease condition of chickens while he was investigating the various causes of "infectious coryza". This we now believe, was *M. gallisepticum*. In 1952, Drs. Markham and Wong (11) investigating chronic respiratory disease in chickens, isolated the first *M. gallisepticum*. This work was carried on in Canada by Drs. Crawley and Fahey *et al* of the Connaught Medical Research Laboratories who isolated the organism, developed the hemagglutination-inhibition test, and made the first proposals for control of chronic respiratory disease in chickens in 1953–57 (8, 9). In Alberta, the first recorded cases of chronic respiratory disease involved six chicken flocks and two turkey flocks in 1952. By 1953, the number of chicken flocks had risen to 90 (2). It is interesting to note that infectious sinusitis had been around longer than we had recognized chronic respiratory disease in chickens. The diagnoses of infectious sinusitis in turkeys at the Alberta Veterinary Laboratory started at least in 1948 (2) and the former poultry commissioner, Mr. Fred Higgenson, indicated that infectious sinusitis had been around for many years before that time. The largest group of respiratory disease problems in chickens in Alberta during 1948, 1949, 1950 was called "infectious coryza". Although isolation of the causative organism of infectious coryza, *Hemophilus gallinarium*, from these cases was attempted before making a definite diagnosis it is possible that *M. gallisepticum* may have been involved.

It was shown by many workers, including Fahey and Crawley (10), that *M. gallisepticum* infection alone, given to healthy birds did not always precipitate the clinical syndrome that is recognized as chronic respiratory disease (CRD). It was also observed that other factors in conjunction with the *M. gallisepticum* were needed to precipitate the typical CRD syndrome. Included in these were various conditions causing stress, e.g. overcrowding, poor ventilation, poor management (such as feed and water starvation), and reactions to vaccinations for infectious bronchitis, Newcastle disease, laryngotracheitis or fowl pox. However, it is now believed that one of the most important factors is concurrent infection with other diseases. This would tie in with the severe reactions for infectious bronchitis, Newcastle disease, laryngotracheitis or fowl pox wherein attenuated live viruses are utilized, bringing about a mild case of the particular disease condition, onto which *M. gallisepticum* infection could be superimposed. Bacterial diseases can also be involved, one of the most common being infection with the pathogenic strains of *Escherichia coli*. The latter has been associated recently with diseases attributed to unfavorable conditions of management, particularly in broiler houses in which large amounts of dust are present in the air (6).

The concept has evolved that *M. gallisepticum* is an organism of moderate pathogenicity which often acts as an opportunist, waiting for other conditions to lower the defences of the bird, then acting synergistically to bring on the typical syndrome of chronic respiratory disease. This results in a prolonged respiratory infection of chickens, characterized by stunting, poor feed conversion and condemnations in broilers, and lowered egg production and condemnations in adults with a comparatively low mortality but high morbidity. Necropsy findings include: caseous material involving the air sacs, frequently pericarditis, perihepatitis, caseation of the lungs, sometimes purulent flakes in the trachea and often in older birds a very rough tracheal mucosa. In turkeys, sinusitis is the most common finding with secondary purulent material in the air sacs, lungs and trachea.

Accurate diagnosis of chronic respiratory disease is not always easy. Three criteria are generally used: (1) the history of a slowly spreading, persistent, chronic respiratory disease, (2) positive *M. gallisepticum* blood test and (3) isolation of *M. gallisepticum*. The latter is the most difficult, as the organism is a very fastidious one and even those who are *septicum* frequently cannot make isolations most experienced in the cultivation of *M. galli-* from all suspected cases.

Research work has shown that *M. gallisepticum* is a fairly fragile organism and very often is not carried over from an old flock to a new flock providing there has been depopulation, a thorough cleanup, and withholding of repopulation for at least two weeks. The organism, however, can be transported in mucous materials from affected birds, on the feet of people, on their clothing or hands, or on dirty egg crates, utensils or equipment. Direct transmission from bird to bird occurs, but this does not result in rapid spread through the flock. The main problem with *M. gallisepticum* transmission is that it can be transmitted in the egg from affected breeder flocks to their progeny, which in turn, infect others in the hatcher or hatchery.

To eliminate chronic respiratory disease, all methods of transmission must be considered. However, particular attack should be made on transmission of *M. gallisepticum* in the egg.

Several programs for the elimination of *M. gallisepticum* have been attempted in Alberta, including concentrating on one breeder flock or concentrating on a group of broiler breeding flocks. These met with negative results. In Saskatchewan, control of the *M. gallisepticum* in all chicken breeding flocks in the province was attempted, again with negative results. Such attempts at control have been tried in other areas in Canada and the United States, but with discouraging results. It would appear that if *M. gallisepticum* is to be eradicated control must be initiated with the primary breeders. If the primary breeders can produce *M. gallisepticum*-free eggs, the industry has a very good opportunity of eliminating this costly disease condition. The primary breeders have been thinking about this for some time and many have already initiated programs to eliminate *M. gallisepticum* from the poultry industry.

One of the tools in eradication of *M. gallisepticum* is a good antigen to detect birds that have been exposed to the organism. For a long time, the difficulty in growing *M. gallisepticum* antigen and standardizing it was the main problem blocking such a control program. Now, aside from a few production problems, *M. gallisepticum* antigen is being produced in large quantities by a standardized method enabling a serological control program to be initiated. Three tests are now in use: serum plate test, tube agglutination test, and hemagglutination-inhibition test. The latter is considered to be the most sensitive, but the most difficult to use on a widespread basis.

Eradication of *M. gallisepticum* in turkeys is well advanced, especially in the United States. For several years a voluntary program for *M. gallisepticum* control by primary breeders has been functioning in which 10% of the blood sera collected for the pullorum test has also been subjected to the *M. gallisepticum* agglutination test. If reactors are found, in most cases the primary breeder eliminates the reacting flock from egg production. To do this, he must have a large number of flocks with the same genetic characteristics and be in a position to eliminate a percentage of these. As a consequence, these breeders can advertise turkeys from *Mycoplasma gallisepticum* (PPLO-S6)-negative birds.

M. gallisepticum control in chickens has been attempted using several methods, some meeting with success, many with failure. The prime objective is to produce eggs free of *M. gallisepticum*. The following have been attempted:

1. (*a*) Test adult birds and eliminate all reactors.
   (*b*) Inject breeding birds with antibiotics to reduce egg transmission.
   (*c*) Divide the progeny into numerous small flocks and raise these separately, with periodic serological testing and elimination of any flocks that show reactors. (This system was proposed by Crawley and Fahey (8) and has been used successfully in the establishment of some *M. gallisepticum*-free flocks.)
2. Immunization of breeding birds by exposing them at a young age to virulent *M. gallisepticum* infection, allowing them to recover and utilizing their eggs for hatching purposes. Some investigators consider this useful, others believe it to be ineffectual (1).
3. Egg dipping. This consists of dipping or injecting an antibiotic into the eggs to control residual *M. gallisepticum* (19, 20). The method holds considerable promise as it does reduce the transmission of *M. gallisepticum* greatly but does not completely eliminate all transmission.
4. The injection of newly hatched birds

with antibiotics. Various combinations of antibiotics have been employed including neomycin, terramycin and tylosin, frequently combined with vitamins of the B complex. Many breeders and growers feel this has reduced mortality in the birds from infectious diseases including *M. gallisepticum*, *M. meleagridis*, *Salmonella spp.* and Arizona paracolon. However, *M. meleagridis*, Arizona paracolon and Salmonella organisms have been isolated in the laboratory from previously injected birds.

5. Heating of eggs. Dr. Harry Yoder of Athens, Georgia, recently reported on a technique for the control of *M. gallisepticum* (20). This consists of superheating hatching eggs to 113° F prior to incubation which, in effect, "pasteurizes" the eggs. In his experiments, the heating eliminated the *M. gallisepticum* with only a limited decrease in hatchability.

If the poultry industry really wants to eliminate *M. gallisepticum* the tools required are now available. A combination of the above methods may be most useful as in the following possible control programs:

Primary Breeder Flock

To reduce egg transmission, a combination of: 1. (*a*) Test the parent flock, and eliminate all reactors or preferably all infected flocks. (*b*) Inject the adult birds with antibiotics (i.e. tylosin or terramycin) at one-month intervals. (*c*) Retest monthly and eliminate reacting flocks. 2. (*a*) Dip or inject eggs with antibiotics (with dipping in tylosin at least 0.3 milligrams of the drug should be taken into the egg and with injection at least 2 milligrams of this antibiotic injected into the egg). (*b*) Heat the eggs prior to incubation to a temperature of 113° F. 3. Raise the resultant progeny in separate groups of 100–500 in each group. Test at one-month intervals and eliminate reacting flocks. (By experience, this may be reduced to one or two tests starting at ten weeks of age.) 4. Following the proposal of Peterson (14), inject chicks with tylosin, 25 mgs/ml in corn oil at day old and at five-day intervals with 0.5, 0.75, 1.0, 1.5, 2.0 ml quantities and also add to the feed, for a 25-day period, chlortetracycline 300 gm/ton and terephthalic acid 0.4%.

Multiplier Flocks

1. Separate hatcher (or hatchery) for *M. gallisepticum*-free eggs.
2. Depopulate six weeks before receiving *M. gallisepticum*-free chicks.
3. Thoroughly clean and disinfect brooder and poultry houses.
4. Screen houses to eliminate wild birds.
5. Trap or eliminate wild birds on premises, especially pheasants, quail, sparrows and starlings.
6. Do not allow visitors into poultry houses unless they wear clean plastic boots and clean coveralls.
7. Do not allow feed trucks or cars into poultry premises or in contact with birds.
8. Do not move equipment from other farms onto clean premises, e.g. crates, feed bags, tractors.

Summary

M. gallisepticum is incriminated as one of the main causes of chronic respiratory disease in chickens and infectious sinusitis in turkeys. By utilizing the tools provided by research, we have within our grasp the opportunity to eradicate *Mycoplasma gallisepticum* from the poultry industry, if we really wish to do so.

Résumé

Mycoplasma gallisepticum est considéré comme étant l'une des causes principales de l'infection respiratoire chronique des poulets et de la sinusite infectieuse des dindes. Si on le veut réellement, il est possible en recourant aux données que nous fournit la recherche, d'éliminer complètement ce germe de l'industrie avicole.

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BOOK REVIEW

The Cultivation of Parasites In Vitro. A. E. R. Taylor and J. R. Baker. Published by Blackwell Scientific Publications, Oxford and Edinburgh and The Ryerson Press, Toronto. 1968. 377 pages. Price $14.50.

In recent years, the emphasis in the study of animal parasites has swung away from the life cycle approach to studies on the behavioral, physiological and biochemical aspects of these organisms. Because of their close association with the host, it becomes imperative, if these studies are to progress, that reliable methods be developed for growing them apart from their hosts. As a result, an impressive amount of information has accumulated on this aspect of parasitology. Although a number of reviews have been written, they have tended to be concerned with specific groups of organisms and have not given a broad coverage of the subject. The work under consideration represents a commendable effort on the part of the authors to collect and condense the multiplicity of literature available on the *in vitro* cultivation of a large variety of animal parasites. It is essentially a compendium of pertinent information and little effort is made to critically appraise the techniques described. The book is divided into three parts. The first two deal with Protozoa and Helminths, respectively. The third part consists of an appendix giving details of techniques of general application.

The presentation is straightforward and the book is easy to read. I found the text to be relatively free of grammatical or typographical errors although, on page 217, Echinococcus was spelled Echinocus and on pages 272 and 325, Marplestone should have been Maplestone. I was most impressed with the very extensive coverage of literature and could find no serious omissions. However, I felt that reference should have been made to the work of Townsley *et al.* (1963) (J. Fish. Res. Bd., Canada 20: 743) on the *in vitro* cultivation of *Terranova decipiens*.

To facilitate reference to the literature, tables of pertinent references are included in each section or chapter. A useful addition for future editions might be a complete author index to all literature cited.

This handbook provides a valuable and much needed source of reference to all research workers in experimental parasitology on the subect of *in vitro* cultivation. *H. C. Gibbs.*