Mycoplasmoses in poultry
L. STIPKOVITS* and I. KEMPF**

Summary: The most important mycoplasmas isolated from domestic avian species include Mycoplasma gallisepticum (MG), M. synoviae (MS), M. meleagridis (MM) and M. iowae (MI). MG causes chronic respiratory disease of chickens and infectious sinusitis in turkeys, resulting in economic losses. MS causes infectious synovitis or mild upper respiratory disease. MM infects only turkeys, causing airsacculitis and sub-optimal production and hatchability. MI is associated with reduced hatchability in turkey flocks. Transmission is either direct, from bird to bird or through the egg, or indirect. Diagnosis is based on isolation and identification of mycoplasmas, according to biochemical, serological or molecular biology tests, or serological examination of host sera by slide agglutination, haemagglutination inhibition or enzyme-linked immunosorbent assay (ELISA) tests. Antibiotics (i.e. tetracyclines, macrolides, quinolones and tiamulin) may be used for therapeutic treatment or prophylactic medication. The eradication of mycoplasma infection can be achieved through improvements in hygiene and management practices, therapeutic treatment of breeder layers and/or of hatching eggs and better monitoring procedures.


INTRODUCTION

Micro-organisms of the class Mollicutes (also called mycoplasmas and, in earlier texts, pleuropneumoniae-like organisms or PPLO) contain both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), lack a cell wall, carry the smallest prokaryotic genome down to 5 x 10^8 kilodaltons (kDa) and have fewer than 300 genes. Mollicutes are divided into two orders: Mycoplasmatales (with two families, Mycoplasmataceae, containing two genera, Mycoplasma and Ureaplasma, and Spiroplasmataceae, containing one genus, Spiroplasma) and Acholeplasmatales (with one family, Acholeplasmataceae, containing one genus, Acholeplasma).

Organisms which have been thought to be avian mycoplasmas were first isolated from chickens in 1935 (43). Subsequently, several serotypes, later classified as species, were cultured, mostly from chickens and turkeys. However, the distribution of mycoplasmas seems to be world-wide in avian species. Poultry specialists are mainly interested in avian mycoplasmas associated with diseases of domestic poultry,
therefore only pathogenic avian mycoplasmas and their diseases will be described in this paper. To date, sixteen species (M. gallisepticum, M. synoviae, M. meleagridis, M. gallinarum, M. iowae, M. iners, M. gallopavonis, M. gallinaceum, M. pullorum, M. lipofaciens, M. glyophilum, M. cloacale, A. laidlawii, A. equifetale, M. imitans and Ureaplasma gallorale) have been isolated from chickens and turkeys and seven species (M. anseris, M. imitans, M. anatis, M. glyophilum, M. lipofaciens, A. axanthum and A. laidlawii) have been cultured from geese and ducks. In addition, several other mycoplasmas and acholeplasma species have been detected in other avian species, such as M. columbinum, M. columbinasale and M. columborale in pigeons.

For veterinary medicine, the most important mycoplasmas which have been isolated from domestic avian species are M. gallisepticum (MG), M. synoviae (MS), M. meleagridis (MM) and M. iowae (MI). Less important are M. anseris, Mycoplasma sp. 1220 (in geese) and Ureaplasma sp. in turkeys and chickens, which cause respiratory disease and reproductive disorders.

Nevertheless, a great effort was made by the majority of international poultry breeding companies to eradicate the main mycoplasma infections, i.e., MG, MS and MM, from primary breeding stocks. There are chronic reservoirs of infection as well as sporadic outbreaks, which appear to be increasing in frequency, even in some developed and regulated countries, such as the United States of America (USA) and those in Europe. The infection rate among parent breeder flocks in some western European countries reaches 20% to 30% (79). In other parts of the world, where the poultry industry is expanding and regulations and diagnostic capacities are inadequate, mycoplasma infections may be widely spread. The term 'mycoplasmosis of poultry' is often used in the literature to describe diseases of chickens and turkeys caused by different mycoplasma species. Diseases caused by different aetiological agents will be described individually in this paper.

**MYCOPLASMA GALLISEPTICUM INFECTION**

MG causes chronic respiratory disease (CRD) of chickens and infectious sinusitis (IS) of turkeys, characterised by rales, coughing, nasal discharge, sinusitis and the development of severe lesions on the air sacs. These diseases are considered to be an important problem in broilers, breeders and commercial layers. Economic losses in the poultry industry caused by this disease are significant. In broilers, there is a reduction in weight gain of up to 20% to 30%, a 10% to 20% decrease in food conversion efficiency, a 5% to 10% mortality rate and 10% to 20% of carcasses are condemned at the processing plant. In breeders and layers, the disease causes a 10% to 20% decrease in egg production (nearly 16 fewer eggs per hen) and a 5% to 10% increase in embryo mortality. As the organism is transmitted through the egg (93), MG-infected breeder flocks should usually be depopulated (55, 71). Moreover, the presence of intercurrent infections, bad housing, overcrowding, poor hygiene and vaccination programmes against infectious bursal disease (IBD), infectious bronchitis (IB), laryngotracheitis and infectious coryza significantly increase economic losses (71).

**Epizootiology**

MG infection occurs mostly in chickens and turkeys. However, this species has been isolated from pheasants, chukar partridge, peacocks, bobwhite quail, Japanese quail,
parrots, wild turkey and ducks (43, 98). Some isolates, which had been cultured from ducks and geese (20, 29), have been described as a separate species, *M. imitans*. Molecular biological studies (DNA homology, polymerase chain reaction [PCR], restriction fragment length polymorphism [RFLP] and hybridisation with a ribosomal gene probe) have confirmed that the two species are genetically related. *MG* infection is readily transmissible to uninfected birds by direct and indirect contact. As *MG* colonises the upper respiratory tract, a large quantity of mycoplasma may be excreted by nasal discharge, breathing or coughing. Transmission depends on the size of the reservoir of infection, the number of susceptible individuals and the distance between them. Transmission may be more likely during the acute phase of infection and is influenced by the ability of the strain to multiply in the respiratory tract (36, 84, 98). Once a bird is infected with *MG*, it is considered chronically infected for life, since the *MG* cells are able to elaborate alternative forms of surface epitopes, to produce spontaneous phenotypic changes in the expression of mycoplasma surface lipoproteins (an antigenic diversity-generating mechanism), to circumvent the host immune response to specific epitopes and to survive in host tissues (101). The incidence of positive isolations may vary from a small percentage up to 70%, but the infection remains for a long time. Thus, infected flocks are often sources of new infections. This is an important point in breeding work when different genetic lines are crossed.

Infected birds are subject to the additional stresses found in the field environment (such as ammonia) and exposure to other micro-organisms (e.g. Newcastle disease virus [NDV], IB, influenza A virus, infectious laryngotracheitis, *Haemophilus paragallinarum* and *Escherichia coli*), which may increase the excretion of *MG* (43, 98). The survival time for *MG* outside the host (in faeces, on cloth and so on) varies from 1 to 14 days and depends upon the ambient temperature and the materials on which the organism resides (98). Therefore, poorly cleaned and disinfected barns or materials can also be sources of infection. It is important to mention that the longest survival time was observed in egg materials (in allantoic fluid: 3 weeks at 5°C, 4 days in the incubator; 6 days at room temperature; in egg yolk: 18 weeks at 37°C or 6 weeks at 20°C). Therefore, egg debris in incubators is essential in spreading infection. It is also interesting to note that *MG* can survive for one to two days on human hair and skin. So, people working with infected flocks can also act as *MG* carriers.

The main route for the spread of *MG* infection, as indicated by many authors, is egg transmission. In the acute phase, *MG* can easily reach the follicles in a high proportion. Later, in infected hens, *MG* colonises the ovaries and oviduct, leading to the laying of infected eggs. Mycoplasma can be isolated not only from the embryos but also from the vitelline membrane of fresh eggs (98). The proportion of infected eggs laid by a flock varies considerably. A proportion of infected embryos die during incubation; a proportion will hatch, carrying the infection to the progeny flock. Consequently, *MG* infection can be transported very long distances by eggs or by one-day-old chicks. In certain cases, the infection could be spread through contaminated living virus vaccine, if such a vaccine was not prepared from specific pathogen-free (SPF) eggs (16). *MG* can be found in the semen of males, so transmission of infection may also occur through artificial insemination in turkeys.

**Aetiology**

Based on a large number of *MG* isolates with different pathological profiles, from different countries, flocks and hosts, a great variation in biological properties, such as infectivity, virulence, tissue proclivity (cerebral arteriotropism) and specificity for
cloacal, joint or eye infection (43, 98, 99, 100), has been noticed, resulting in very
great variations in epidemiological, clinical and pathomorphological pictures. Soeripto
et al. reported on strains causing severe air sac lesions or substantial loss of egg
production over a five-week period, following their injection into the abdominal air
sacs of chickens (84), as well as on strains which did not produce gross lesions or loss
of egg production. However, the slightly virulent strains produced clinical
conjunctivitis in combination with IB virus (IBV) strains. A combination of agents also
influenced the presence of MG in tracheal washings. Some strains spread very quickly
by contact, inducing a serological response in contact chickens in as little as four
weeks, while other strains spread very slowly, producing a serological reaction after
sixteen weeks. Differences were also observed in egg transmission. Consequently, the
disease may range from very mild to very severe; it can spread slowly or very quickly,
and it can either be diagnosed easily by isolation of typical strains and/or
demonstration of a strong serological response, or the diagnostic procedure may be
very difficult because of the isolation of so-called variant (atypical) strains (96), or the
development of a very poor serological response. Certain isolates of MG have become
more commonly known by their designation: the strain S6 was isolated from the brain
of a turkey with infectious sinusitis; strain A5969 became a standard strain for antigen
preparation; strain R, which was isolated from a chicken with airsacculitis, is used for
challenge and bacterin production and strain F is commonly used in live vaccination
programmes (58, 59, 65, 98, 99, 100, 101).

In pathogenesis, such factors as mycoplasma neuraminidase, peroxidase or other
haemolysins, lysosomal enzymes and exotoxins may result in cell damage. Mycoplasmas attach to the plasma membrane of epithelial cells of the respiratory tract
(nasal cavity, trachea, lung and air sacs) by a terminal structure known as the bleb. The
receptor sites on the host cell membrane are sialic acid residues of sialoglycoproteins.
Mycoplasmas are in close apposition to epithelial cells and collagen fibres of the
lamina propria. This leads to deciliation and degeneration of cells (enlargement of
mitochondria and endoplasmic reticulum) with desquamation of the epithelium. In
addition, there may also be an inflammatory and immune response, since infiltration
of the subepithelial tissue with monocytes, large numbers of lymphocytes and
lymphofollicular aggregations (containing germinal centres indicating a bursa-
dependent immune response) has been observed. This results in marked thickening of
the mucosal membrane of affected tissue (infiltration) and hyperplasia of the mucous
glands, submucosa and pneumatic areas (43, 98). An immune mechanism may also
be responsible for mycoplasma antigen in the glomeruli, since a high concentration of
immunoglobulin G (IgG) was found in the same area, and for massive invasion of the
joint by lymphocytes. It has been suggested that toxins may be responsible for acute
encephalopathy, presumably by causing increased permeability of the vessels, swelling
of the endothelium, fibroid changes and infiltration of the vessels with small round
cells. Exacerbation of the disease occurs with certain associated infections, probably
because invading viruses or E. coli cause cell damage, releasing lysosomal enzymes
which further enhance penetration of mycoplasmas.

Recent advances in methodology, especially in molecular genetics technology, are
applied to the assessment of genetic relatedness among strains and solving the problem
of distinguishing MG strains with different biological properties. Such techniques
include the comparison of electrophoretic patterns of mycoplasmal DNA of tested
strains digested by restriction nucleases (57, 58, 59), and Southern hybridisation of
mycoplasmal DNA digested by restriction enzymes (Bgl II or Hind III), with the probe
pMC5 containing the highly conserved ribosomal ribonucleic acid (rRNA) genes of *M. capricolum* (99, 100). Basically, there are three clusters of MG strains, as follows.

The first cluster includes strains which are serologically identical, but differ in virulence. The most prominent example is the naturally attenuated F strain, which is used as vaccine. These strains are considered non-pathogenic for chickens. However, they can cause clinical disease in turkey breeder hens and in meat turkeys (65). This cluster contains the virulent strain R and strain A5969, frequently used for experimental challenge. Using genomic fingerprints, however, F strains can easily be distinguished from the virulent MG strains (59, 99). Minor but distinct differences between the F strain and virulent strains were also demonstrated by detecting a distinct band in the F strain, slightly above the 68 kDa marker, in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns (52), and by observing electrophoretic patterns of DNA digested by endonucleases, by DNA-DNA hybridisation and by PCR (57, 76).

The second cluster includes variant, atypical strains, such as strains 503, Y5, Y9, M876 and M35, which show deletion of a protein band between the 35 and 43 kDa levels. The main features of such strains are: reduced ability to elicit a typical serological response, reduced antigen interaction with antibodies to the standard strain and a less marked clinical response. Moreover, serological behaviour within the group is different but the fingerprint is unique (57, 59, 99).

The third cluster is composed of strains isolated from hosts other than chickens and turkeys (such as geese, ducks and partridge) and again showing a distinct pattern (57, 58, 99).

Clinical signs

Under natural conditions, the incubation period may vary considerably (from three to 38 weeks) (25). In flocks infected through eggs, clinical signs may develop at the age of three to six weeks in some cases or, in other cases, only near the onset of egg production. In the case of flocks hatched from eggs dipped in antibiotic solutions to control MG, in good hygienic conditions, signs may not appear until some associated disease or stress factor occurs. The most common clinical signs are nasal discharge, tracheal rales, coughing, sneezing and swelling of one or both infra-orbital sinuses (mostly in turkeys) and mild conjunctivitis. Appetite remains near normal as long as the birds can eat. Sometimes, ataxia, lameness, swelling of the hock and enlargement of the eyeballs are observed. Non-specific signs, such as a reduction in growth rate and egg production and increased feed conversion efficiency are common. Clinical symptoms are generally more severe in males than in females, and turkeys appear more diseased than chickens. Morbidity varies depending on age (young birds are more severely affected than older ones) and on ambient temperature (in the cold, the disease is more severe and of longer duration). Complicated CRD (i.e. air sac disease due to other agents, such as *E. coli*) is encountered more commonly in the field. The mortality can be low in uncomplicated disease but may reach 30% in complicated outbreaks (43, 98).

Lesions

Lesions include an excess of mucus, catarrhal exudate in the nares, sinuses, trachea, bronchi, lungs and air sacs, oedema of the air sac walls and caseous exudate in the air
sacs and in the oviduct. In complicated cases, pericarditis, perihepatitis and, sometimes, swelling and oedema of peri-articular tissue, excess joint fluid, erosion of the articular surface (arthritis), inflammation of tendovaginal sheaths, bursae and the synovial membrane (synovitis), and pale areas in the cerebrum (vasculitis) may be observed (43, 98).

**Diagnosis**

Respiratory clinical signs and pathomorphological lesions of the respiratory tract are not pathognomonic for *MG* infection. Diagnosis of *MG* infection requires laboratory confirmation, which may employ different approaches.

**Isolation and identification of Mycoplasma gallisepticum**

*MG* can be isolated principally from the respiratory system (air sac, turbinate, lungs, sinus and choanal cleft) or reproductive tract (oviduct and ovaries), testicles and cloaca, as well as from many other organs. *MG* can be present in bile, too. For isolation, Frey’s medium (31) or medium B (28) are the most frequently used. Attention should be paid to the type of swabs used for sample collection (102). Since several mycoplasma species may colonise chickens and turkeys, mycoplasma isolation often results in a mixture of mycoplasma species. Therefore, isolates should be further examined and identified. At present, epi-immunofluorescence (24) or immunoperoxidase techniques are the most convenient methods for the identification of *MG* in mixed culture on primary isolation plates. The application of direct immunofluorescence by using fluorescein isothiocyanate-labelled antibodies to *MG* and contrasting tetramethylrhodamine isothiocyanate-labelled antibodies to other species, such as *MS*, makes it possible to identify two species in one step. Use of a combination of direct immunofluorescence and immunoperoxidase staining (applying peroxidase-labelled antibodies against a third species, like *MM*) facilitates the rapid detection of three species at the same time (14). This method is very useful for examination of such hosts as turkeys, which can carry three pathogenic species. If isolates cannot be identified, further examinations should be made. Isolates should be filter-cloned at least three times and identified by some of the tests described below.

**Biochemical examination**

Fermentation of glucose, hydrolysis of arginine, reduction of tetrazolium, phosphatase activity, film and spot production and haemagglutination of chicken or turkey erythrocytes are the most characteristic properties (43, 85).

**Serological tests**

It should be kept in mind that some proteins of *MG* are serologically related to proteins of other mycoplasma species, such as *MS*. Therefore, cross-reactions may occur in serological tests. However, the following tests are used most frequently for the identification of *MG* strains:

a) Growth inhibition

Use of a solid medium inoculated with the isolate to be identified. Around a paper disc soaked with anti-*MG* hyperimmune sera, a 2 mm to 10 mm inhibition zone can be seen.
b) Growth precipitation

Solid plates are inoculated with the isolate to be identified and a hole, made in agar, is filled with hyperimmune serum against MG. A growth-inhibition zone and precipitation lines can be observed around the hole.

c) Examination of colonies by antiserum

Polyclonal or species-specific monoclonal antibodies (MAbs) (72), labelled with fluorescein isothiocyanate, tetramethylrhodamine isothiocyanate or peroxidase, may be used for indirect, direct and combined methods (see above; 14, 24). A MAb 6F10 has been developed, which stains predominantly the F strain of MG colonies (and also stains colonies of strains R, S6 or A5969 less frequently, but not others). However, since there is a significant variation in the expression of epitopes, some clones may be stained only partially or not at all (27, 32, 33).

d) Haemagglutination-inhibition test

Broth culture of the isolate is tested for haemagglutination (MG agglutinates chicken or turkey erythrocytes in a dilution of 1:2 to 1:64). Anti-MG hyperimmune serum inhibits haemagglutination performed with four haemagglutination units in a dilution of 1:4 to 1:256 (58).

e) Metabolic inhibition test

Broth medium is inoculated with the isolate and incubated with various dilutions of hyperimmune serum prepared against MG. This may inhibit glucose metabolism in test broth culture inoculated with $10^3$ colony forming units (CFU) or $10^2$ colour change units (CCU) in dilutions from 1:1,000 to 1:20,000.

f) Enzyme-linked immunosorbent assay

Antigen prepared from the test isolate is coated on a polystyrene plate and tested with dilutions of MG hyperimmune serum. The serum can bind to MG antigen in the enzyme-linked immunosorbent assay (ELISA) in dilutions of 1:500 to 1:10,000.

g) Immunoblot (Western blot) analysis

Proteins prepared from the strain are separated by SDS-PAGE, transferred to nitrocellulose (92) and treated with polyclonal hyperimmune or convalescent sera or MAbs. The antigen-antibody reaction is demonstrated by using labelled (with peroxidase or phosphatase or other) antiserum (prepared against IgG of the serum applied in the first step) and developing solution (e.g. 4-chloro-1-naphtol and hydrogen peroxide). It should be kept in mind that unabsorbed polyclonal rabbit antisera raised against MG not only detect species-specific proteins of MG but can also react with several proteins of MS (such as p124, p76, p51, p44 and p36), MM and MI (p205). Antisera against other species can react with proteins of MG (p36). Therefore, hyperimmune antisera previously adsorbed with proteins from other mycoplasma species should be used. It is interesting to note that Western blot analysis based on the reaction of MS antiserum with a protein profile of MG revealed three clusters: one of typical chicken strains (e.g. strains R and A5969), another of strains isolated from
other hosts (i.e. strain 4229 from ducks or strain 30902 from geese) and one cluster of variant MG strains (such as 503 or 730) (100).

More reliable results can be obtained by using MAbS (15, 27, 69), for example, MAb G46 reacting with a protein of 110 kDa, stable to heat and iodate oxidation and sensitive to pronase, located on the surface of MG (41), or MAb G9 (78) reacting with p90-98 kDa of MG but not with other species, or MAb G12 recognising p100 in all MG strains. An important protein of MG is p64, which is partially trypsin-sensitive and has a role in attachment to the tracheal rings (since anti-p64 polyclonal and monoclonal antibodies strongly inhibit attachment, growth and uptake of $^3$H-thymidine of the strain), but does not haemagglutinate. This protein is strongly expressed in strains of high virulence but not in strains of low virulence (8).

It was demonstrated that MG contains size-variant membrane-associated proteins ranging from 44 to 55 kDa, detected by MAb 1E5 common to VspA and VspB of M. bovis (101). These integral membrane proteins separated exclusively into the hydrophobic phase of TX-114. In addition, a surface protein of 41 kDa separated into the aqueous phase was also recognised in most of the MG strains. Using this MAb, it was demonstrated that this epitope-bearing protein is also surface exposed and subjected to high-frequency phase variation. Furthermore, proteins p67, p72 and p75 are also involved in high-frequency surface variation. It was possible to distinguish strains R and F by using sera of chickens infected with strain R and collected two weeks post-challenge. These sera reacted with p75 of the R strain, and with p80 in the F strain. The late response (sera were collected 8 to 11 weeks post-challenge) recognised the p130 and the protein which is slightly heavier than p200 kDa in the R strain but not in the F strain.

Other tests

a) Sodium dodecyl sulfate polyacrylamide gel electrophoresis

The principle of this method is that proteins prepared from MG are treated with SDS and separated in polyacrylamide gels by electrophoresis (63). The polypeptide bands ranged between 30 kDa and 140 kDa are most important. This technique is useful for differentiation of strains of different species or even identifying strains within MG species. It is possible to distinguish F vaccine strains from virulent strains by a prominent band at approximately 75 kDa and a less prominent band at approximately 64 kDa (65), or by a distinct band slightly above 68 kDa (52), or by the different pattern in the range of 92.5 to 200 kDa (9).

b) Electrophoretic pattern of DNA digested by various endonucleases (restriction fragment length polymorphism analysis)

By this method, DNA digested by Eco RI and Bam HI resulted in a considerable homology of MG strains which was different from that of other species. At the same time, strain S6 and virulent R strains were sufficiently distinct from vaccine F strains (53, 54, 57, 58, 59, 65, 83). Therefore, this technique is useful for epidemiological studies of infection.

c) Deoxyribonucleic acid probe and polymerase chain reaction

DNA probes have been developed in several laboratories (42, 48, 49, 54, 66, 82, 90). In this approach, previously purified and radioactively or non-radioactively labelled
MG-specific DNA hybridises with the DNA of the strains to be tested. This technique is rather insensitive and requires approximately $10^6$ cells. In PCR, two primers, previously selected from MG-specific DNA sequences, are used to amplify a small amount of MG nucleic acid to a level that can easily be detected by the DNA probe [76]. Recently, a commercial flock checking DNA test kit became available. It is designed to be specific for the MG strain and can differentiate the F strain from non-F strains of MG. This test enables detection of a minimum of 100 micro-organisms within two days.

d) Combination of polymerase chain reaction, restriction fragment length polymorphism and ribosomal gene probe

DNA from different strains, digested with Hind III or Eco RI endonucleases, is submitted to Southern hybridisation with a probe containing plasmid pMC5, carrying the 5S, 23S and part of the 16S gene of one of the rRNA operons of M. capricolum (75, 99, 100). F strain, virulent R and A5969 strains of MG and MG strains from hosts other than chickens and turkeys can be distinguished in patterns digested by Bgl II and Hind III. For mycoplasma 16S rRNA sequence analysis, oligonucleotide primers M16SPCR5' and M16SPCR3', consisting of 17 nucleotide sequences (sequence: 5' AGGCAGCAGTAGGGAAT 3' and 5' CGTTCTCGGGTCTTGTA 3', respectively), common to all avian mycoplasma species and complementary to the conserved regions U2 and U5 of 16S rRNA of the species, were selected. The selection was based on information about 16S rRNA of MG and other species, obtained from GenBank and used for amplifying 16S rRNA of different species. The PCR product (1026 bp) was digested with restriction endonucleases (Hpa I, Hha I, Hae III, Hph I, Fok I and Mla IV) and electrophoretically examined. MG (and some other species, such as M. gallinarum, M. pullorum and A. laidlawii) can be identified by their unique RFLP of PCR product digested by Hpa I. M. cloacale can be distinguished from MS by RFLP analysis of PCR product digested by Hha I, and from M. lipofaciens after digestion by Hae III.

Direct detection of Mycoplasma gallisepticum in tissue

Deoxyribonucleic acid probe and polymerase chain reaction

A great effort was made to develop a diagnostic method for the rapid detection of a specific agent directly within a clinical specimen, in sufficient time to influence control of the infection. This resulted in the development of DNA probes and PCR. The test is performed in essentially the same way as with culture but using clinical samples. Sántha et al. (83), Hyman et al. (42) and Kempf et al. (48) reported the isolation and characterisation of species-specific clones, generated from the partial genomic library of MG, which were labelled with phosphorus ($^{32}$P) by random priming or with biotin by nick translation, which could detect approximately $10^5$ CFU of MG. Consequently, this method was found to be acceptable in the acute stage of the infection. However, amplifying MG DNA by PCR, using, for example, MG 16S rRNA specific primers producing a specific product of 330 bp (49), and then hybridising this DNA with a digoxigenin-labelled probe, gave much better results. The positive detection rate proved to be 97% of experimentally infected birds, in comparison with MG culture which detected 67% of birds, and the sensitivity of the method was less than one CFU/ml. Similar results were obtained by other authors (76). The advantages
of PCR are that the test is not adversely affected by contaminant organisms or by overgrowth of non-MG strains or absence of growth of MG. PCR allows the testing of composted or pooled samples and, according to the selected primers, it may be possible to distinguish virulent MG strains from vaccine F strains, as indicated earlier.

Capture enzyme-linked immunosorbent assay

A MAb (Myc-9) reacting with MG, MS, MM, MI, M. anatis and M. gallinarum was developed and is used to coat microtitre plates. Broth medium is distributed in coated wells, inoculated with specimens or strains and incubated for one to three days. After discarding the fluid, plates are treated with N-octyl glucosid. Then IgG from antisera against MG or other species adsorbed with heterologous antigens is added. Peroxidase-conjugated goat anti-rabbit IgG is used for visualisation of the reaction. The test seems promising but further studies are necessary to evaluate the test for examination of field specimens (1).

Serological examination of host sera

In routine work, detection of MG infection may be achieved by detecting antibodies against MG in the host organism. For this purpose, various tests are used, as follows:

Serum plate agglutination

One drop of stained MG antigen is mixed with one drop of host serum. Clumping of stained antigen with clearing of the suspension constitutes a positive reaction. Agglutination develops in two minutes. This test measures primary IgM (81). It is widely used and is very simple and sensitive. However, many non-specific reactions may occur, depending on several factors, such as the strains and media used for antigen preparation (antigens from different companies may show various sensitivities and specificities), the quality of sera, the hosts from which sera were obtained, the antigenic relationships with other species (MG antigen may react with MS antisera, for example), or the strains involved in infection (98). Using oil-emulsion vaccines against fowl coryza or inactivated IBDV vaccine or other vaccines produces a strong systemic antibody response to components of mammalian sera (4), and these antibodies probably react with serum components from the broth medium associated with MG cells during growth, causing false positive reactions. These reactions can be seen two to five weeks post-vaccination. They cannot be prevented by heat inactivation of sera or treatment by 2-mercaptoethanol, dithiothreitol or 3 M sodium chloride (97). The serological response induced by so-called variant strains demonstrates positive in serum plate agglutination (SPA) in only 20% to 40% of chickens (96).

Haemagglutination-inhibition

Broth culture in the log phase of MG or cell suspension or lectin-purified protein of MG (21), in a determined concentration, is mixed with dilutions of the sera to be tested. Then fresh or formalinised chicken red blood cells are added. Inhibition of hemagglutination indicates the presence of antibodies against MG. The test can be performed both with serum and saline or chloroform-extracted yolk from fresh eggs. The test primarily measures IgG (81), and antibodies detected with this test persist for several months. This test is very specific (no cross-reaction is observed with antisera against MS or other mycoplasma species), but rather insensitive.
inhibition (HI) showed a wide antigenic diversity which could not be detected with restriction enzyme analysis (REA) (58, 67, 68). HI titres depend on the strain used in performing the test. This test cannot detect a serological response induced by variant MG strains. Nevertheless, a combined use of SPA and HI gives important information on MG infection, as follows:

- low positivity in SPA (below 30%) and 3% to 10% positivity in HI indicates fresh MG infection
- high positivity in both tests indicates infection which started three to eight weeks previously
- low SPA positivity and high positivity in HI demonstrates an infection which began three to six months previously.

According to some authors, a polypeptide of 69 kDa constitutes all or part of haemagglutinin (18), which can be specifically detected by MAb E-12. According to Barbour et al. (9), an electro-eluated protein of MG R strain, namely, adhesin (p75 kDa), showed haemagglutination activity on chicken erythrocytes. With the appearance of antibodies specific to p75 and p60, there was a significant rise in the HI geometric mean titre of chicken sera.

Using immuno-affinity chromatography with MAb 86, Markham et al. purified a polypeptide p67 (pMGA), to be located on the cell surface (67). MAb 86 and 71 reacted with a single band of the p67 polypeptide of MG S6 and R strains, and with a single band of p75 of MG F strain, but MAb 66 failed to react with either the F or R strains, demonstrating a different expression of epitopes. MAb 86 and 71 inhibited haemagglutination, supporting the proposal that pMGA is a principal haemagglutinin of MG. Using electrophoretic purification, Avakian et al. isolated a p64 polypeptide (6), which did not haemagglutinate chicken or turkey erythrocytes but reacted with MAb 86, described by Markham et al. (67), suggesting that p64, p67 and p69 are identical, and that differences in molecular weight are due to experimental errors, or to the expression of different genes of antigenically related products of different size (68).

However, Forsyth et al. (30) purified, by electro-elution from PAGE, a surface-exposed integral membrane component lipoprotein p64, localised to the base of the terminal structure (tip) of MG. The anti-lipoprotein p64 antibodies inhibited haemagglutination of chicken erythrocytes and attachment of MG to the tracheal epithelium. This antibody did not react with MS and did not inhibit haemagglutination due to MS.

**Enzyme-linked immunosorbet assay**

Microtitre plates (or nitrocellulose membrane in dot ELISA) are sensitised by MG antigen and treated with dilutions of the sera to be tested. The antigen-antibody reaction is visualised by adding antiserum directed against IgG of the sera to be tested and enzymes. As the ELISA test detects mainly IgG antibodies, a positive reaction can be demonstrated for a much longer time post-infection than with SPA. The broad acceptance of ELISA has been due to the sensitivity, ease of performance and automatisation of the testing procedure. Great attention should be paid to the antigen concentration, antibody dilution, antibody-antigen incubation time and the time at which the reading is taken after stopping the reaction. Several ELISA kits are available (3). This test is much more sensitive than HI. Early kits gave many non-specific
reactions, as with SPA, especially in flocks immunised with various oil-based vaccines of tissue culture origin and reacted with sera obtained from MS-infected birds (18). However, many efforts were made to eliminate this reaction by purifying antigen with Triton X-100 (77), lectin (18) and immunostimulation complex (ISCOM) (91). Species-specific proteins (i.e. p64, p56 and p26 kDa) prepared as antigens for ELISA proved to be sensitive and specific in dot-ELISA (4, 5).

**Blocking enzyme-linked immunosorbent assay**

Microtitre plates are coated with MG antigen and treated with the undiluted sera to be tested. A peroxidase-labelled MAb, B3 (directed against p56), is added and visualised by enzymes, as in the other ELISA test (23). A colour reaction indicates negative results, whereas the absence of colour indicates the presence of MG antibodies. In chickens challenged below three weeks of age, the blocking ELISA demonstrates positive results one week later than SPA. In older birds it demonstrates such results at the same time as SPA, since anti-p56 antibodies in young birds appear in the second week post-challenge. The sensitivity of this test is the same as that of the indirect ELISA kit (51). The test recognises 50% more positive birds in an infected group than HI, whereas all HI-positive birds also gave positive results in the blocking ELISA (23). The blocking ELISA can be used for testing both sera and eggs. The great advantages are as follows:

i) the test uses the MAb B3 prepared against p56, one of the most stable, species-specific proteins, expressed in almost all MG strains tested up to the present (7) and inducing a strong immune response in chickens and turkeys

ii) the sera to be tested can be used undiluted or pooled

iii) the test is not host species-specific and can be used not only for testing chickens but also for turkeys and any other animal species. Thus it can be used to detect MG infection in any other birds, including wild birds, which might be incriminated in the spread of MG infection (47)

iv) the test can also detect the chicken antibody response induced by variant MG strains (22).

**Immunoblotting**

A nitrocellulose membrane containing MG protein transferred from SDS-PAGE is treated with sera from infected birds (see above). Sera from MG-infected hosts react with most species-specific immunogenic polypeptides of MG, such as p139, p120, p76 and p69 (18), or p85, p64, p56 and p26 (but they can also react with some polypeptides of MS, e.g. p88 and p53 kDa) (5).

Sera obtained from chickens challenged with the virulent R strain react weakly to proteins of variant strains, such as strains 236, 383, 503, 703, 730 and K1669, showing considerable antigenic differences. Sera from turkeys infected with the variant strain M876 react differently to strain S6 (7). Species-specific protein p64 could be detected in most MG strains when using hyperimmune serum, but only in half of these strains using convalescent sera. In contrast, p56 is consistently present in most MG strains and induces a good response in chickens and turkeys, even in the case of a variant MG infection. P26 was evident only in about 70 strains (6) and does not appear to be immunogenic in turkeys (7). According to the experience of the authors, this technique can be useful in detecting antibody response to MG. However, it is important to take
into consideration the age of the chickens tested and the time post-challenge. In chickens younger than three weeks, antibodies to p64 and p67 develop in the first week post-challenge while antibodies to p56 can be detected in the second week post-challenge. However, in older birds, antibodies to these immunogenic proteins can already be recognised one week post-challenge. Antibodies to p85, p35, p26 and p24 can be observed much later.

**MYCOPLASMA SYNOVIAE INFECTION**

*MS* infection causes infectious synovitis and sometimes upper respiratory disease of chickens and turkeys, especially when *MS* infection is combined with Newcastle disease (ND), IB infections or vaccination (43, 60). *MS* has a significant economic impact on broilers. Studies on the effect of *MS* on layers are contradictory. According to some authors, *MS* infection may cause a drop in egg production of up to 10 eggs fewer per hen (71). However, this difference disappeared when hygiene, flock density and vaccination programmes were considered. According to the experience of the authors, a reduction in egg production of 5% to 10% and a reduction in hatchability of 5% to 7%, with more than 5% mortality in the offspring flock, was observed in *MS*-infected breeder flocks without obvious clinical symptoms.

Difficulties in the isolation and serological detection of *MS* infection and in the reproduction of the disease with some isolates, due to the great variation in the virulence and antigenic properties of *MS* strains, led to the opinion that *MS* was not important. Consequently, less attention was paid to control programmes, resulting in a significant spread of *MS* infection during the last few years.

**Epizootiology**

*MS* occurs mainly in chickens and turkeys (43, 60). However, *MS* has also been isolated from guinea fowl, ducks, geese, pigeons, Japanese quail, pheasants and house sparrows. Moreover, these birds are sensitive to artificial infection, too. This infection is widely spread and the occurrence is increasing. Like *MG* infection, *MS* spreads by direct and indirect contact, as well as by egg transmission, in which the highest rate is in the first four to six weeks after infection, and so on, but this disease spreads more rapidly than *MG* infections. *MS* is common in multiple-age farms. Exacerbating factors may modify the excretion and transmission of *MS*. An important point in epidemiology is that great variations occur among *MS* strains in virulence and tissue tropism, leading to various forms of the disease. Genetic conditions, age, immune status of the host and intercurrent infections with IB, NDV, influenza A, *E. coli*, *MG* and *MM* act synergistically and modify the spread of *MS* infection. As with *MG*, *MS* can be isolated from bile.

**Aetiology**

There are great variations among *MS* strains in virulence and tropism for joints or the respiratory tract. However, these differences cannot be detected by serological examination. There is little difference in pathogenicity between haemagglutinating and non-haemagglutinating strains of *MS*. In regard to pathogenesis of the disease, the number of penetrating organisms and the route of infection are important. Foot
penetration produces synovitis, while penetration of MS into the air sac (by aerosol) causes airsacculitis. These differences can be detected by Western blot analysis and DNA studies of strains (99) (see 'Diagnosis'). Infection through the egg produces both types of disease. Pathogenesis of MS infection is mediated by the same mechanisms as shown for MG. However, in MS infection, anaemia, which develops through damage of the erythrocytes, is a very important feature. Localisation of MS in tissue and its metabolic products attract neutrophils which, when damaged, release lysosomes. This may explain the progressive accumulation of caseous materials around tendons and bursae and in joints. These lesions also explain the infiltration of small round cells, plasma cells and macrophages into damaged areas (in joints or in air sacs). In this process, a thymus-dependent cell-mediated response is involved. Airsacculitis is very much exacerbated by vaccination against ND or IB or the presence of IBD, and is greatly enhanced by environmental temperature.

It should be remembered that there is strong serological (3) and genetic (99, 100) relatedness between MS and MG strains, which hampers identification of MS strains and the diagnosis of MS infection.

Clinical signs

The incubation period also varies greatly, depending on the route of infection, number and virulence of organisms, susceptibility of the host and existence of associating factors. In birds infected by egg transmission, incubation lasts about 6 weeks; in those infected by contact: 11-21 days; after foot pad infection: 2-10 days; by intravenous infection: 7-10 days; by intrasinus infection: 7-14 days; by conjunctival instillation: 20 days. (Antibodies can be detected before clinical disease becomes evident [43, 60].) The disease has two forms, as follows.

Lameness with or without generalisation

This form has been observed mainly in the past, 20 to 30 years ago. Morbidity was approximately 5% to 15%, sometimes more; mortality was 1% to 10%. Clinical symptoms were as follows: depression, poor growth, paleness of the face and comb, lameness, swollen hock joint and bursae, ruffled feathers, green diarrhoea and droppings containing a large amount of uric acid or ureates.

Respiratory form

The respiratory form of MS is common nowadays. The infection occurs most frequently as a subclinical upper respiratory disease that may become systemic. The clinical signs are similar to those of MG infection: respiratory signs, lameness, retarded growth, increased mortality and variable decreased egg production (60). Once again, turkeys can be more severely diseased than chickens but respiratory signs are not usually observed in turkeys.

Lesions

Thickening and oedema of the joints with exudate, erosions of articular cartilage (arthritis and tendovaginitis), hepatomegaly, splenomegaly, swollen pale kidneys and atrophy of the bursae Fabricius and thymus are the most important lesions. Lesions of the respiratory tract are similar to those observed in MG infection, but milder (43).
Diagnosis

Signs and lesions are not pathognomonic. Diagnosis of MS infection should be conducted in a similar fashion to that of MG infection. In the isolation procedure, it should be taken into consideration that MS requires a medium containing nicotinamide-adenine dinucleotide (NAD) and cystein (31). It was demonstrated that plain or charcoal cotton swabs on wooden or plastic sticks were more likely to produce growth if retained in the medium for incubation, while rayon swabs on aluminium wire caused inhibition of growth (102). Recovery of MS from the synoviae, bursal or joint lesions of chronically affected birds is difficult, and sampling of the respiratory tract is more reliable. MS can be isolated from flocks with negative SPA (64). When using biochemical and serological testing to identify strains, similar principles should be followed to those used in the identification of MG. A new technique, coagglutination assay (73), was developed using MAbs for rapid laboratory identification of MS strains. A MAb S2 (IgG3 isotype), agglutinating MS, which binds to p55 kDa protein, and sometimes to p11 and p75 of MS (but not to proteins of MG), was coated on Staphylococcus aureus (Cowan 1 strain), containing protein A. Mixing with MS culture resulted in coagglutination of MS strains. The test was specific for MS.

The electrophoretic pattern of MS DNA when digested by Eco RI and Hind III revealed marked homology and a characteristic profile for MS, but Bgl II-digested patterns indicated some variations, dividing the MS strains into clusters of:

- arthrogenic strains (such as the WVU 1853 and Olson strains)
- an air sac virulent strain (K1415) (99).

The usefulness of applying Bgl II was confirmed by Ley and Avakian (64) in epidemiological studies of MS strains. They could prove homology of strains of geographically different origins in addition to variability of strains. Protein and restricted DNA patterns of individual isolates were highly reproducible and remained unchanged following long-term passages of strains (400 generations).

MS does not induce a strong immune response. It is well known that environmental conditions, the presence of avian respiratory viruses and tissue tropism of MS can profoundly affect not only the type and severity of disease caused by this agent (43, 60) but probably the immune response also. To detect a serological response due to MS, SPA, HI and ELISA tests can be performed with the same precautions as described for MG (non-specific reactions may occur after vaccination with oil-emulsion vaccines; moreover, after MG infection, these tests may not be effective in turkey flocks). Antibodies can be detected by HI and ELISA (not by SPA), not only in sera but also in egg yolk. In naturally infected chickens, IgG antibodies against MS can be detected in the Harderian gland, hock joint and bursa Fabricius (11, 13), oviduct and egg albumen (IgA and IgM). The progeny of infected parents convert to serologically positive by 8 to 12 weeks of age. In immunoblotting, it was proved that p53 and p22 are species-specific proteins of MS (p53 is immunogenic only in the acute phase), whereas p41 is non-specific (5). Recently, p22 and p92 kDa and proteins with molecular masses from 46 to 52 kDa, partitioning into the detergent phase of Triton X-114 lysates, and purified by ion-exchange chromatography, proved to be specific antigens for ELISA (38). It has been reported that MS induces cell-mediated immunity, producing positive reactions in the leucocyte migration inhibition assay and skin test (13). The possibility was also suggested that MS exhibits an inhibitory influence on the development of MG humoral antibody (60). Bencina et al. (12, 13) detected...
considerably higher rates and titres of MS antibodies (mostly IgG and IgA) in biliary samples than in the same assay performed with sera. In many cases, sera of chickens may be negative for antibody while their bile gives positive results. DNA probes were developed to detect MS in clinical samples (42, 48, 105). However, once again, a combination of PCR with probe seems to be much more sensitive than the probe alone.

**MYCOPLASMA MELEAGRIDIS INFECTION**

*Mycoplasma meleagridis* (*MM*) infects only turkeys, causing airsacculitis in young flocks, a reduction in food conversion, sub-optimal production and hatchability and downgrading of carcasses and day-old poults.

**Epizootiology**

In the past, the infection occurred in almost all turkey flocks. Reports concerning the occurrence of *MM* in Japanese quail, peacocks and pigeons were not confirmed (94). Owing to an intensive eradication programme, the occurrence of infection has decreased. Direct (by the air-borne route within the hatchery and flock, and between flocks separated by 1/4 mile) and indirect transmission (by sexing, palpation of hens, vaccination or contaminated hands, clothing and equipment) may occur at any time during the life of the bird. Factors influencing the spread of *MM* infection are similar to those of *MG*; however, the lateral spread of *MM* infection is of little significance. *MM* has tropism for the cloaca and bursa Fabricius in young stock and for the phallus and oviduct in adults. Therefore, artificial insemination plays a more important role in the spread of the disease. Factors influencing the infection are also important. Age (lesions in air sacs tend to be limited to birds under 14 weeks of age) and intercurrent infections have a minor role but synergism exists between *MM* and *E. coli* or *MS* infection. Egg transmission of *MM* is more important. Egg transmission may vary from 10% to 60% (43, 94). Transmission is less frequent at the beginning and at the end of the laying period, and more frequent at midseason. Egg transmission may lead to active airsacculitis, and such transmission may not be observed in hens with upper respiratory tract lesions. *MM* may be present in the oviduct, uterus, magnum, vagina (19%-57%), cloaca (but not in the gonads or ovules), shell membrane, vitelline membrane (10%-12%), feathers, skin, sinus trachea, lung, bursa Fabricius and intestine. The isolation rate from the phallus and semen may vary from 13% to 32%.

**Aetiology**

Variations in virulence in *MM* strains and tropism for the cloaca and bursa Fabricius in young stock and the phallus and oviduct in adults have been observed. Variations have also been reported in the invasive properties of *MM* strains (43, 94). These differences were not detectable by cross agglutination, immunofluorescence or immunodiffusion but strains differed in their SDS-PAGE patterns (103). *MM* invades organs of the respiratory, intestinal, reproductive and skeletal systems of the turkey. The route of infection is also important since airsacculitis and osteodystrophy are most likely to follow infection of embryos. Little is known of the pathogenesis of *MM* infection. In the development of air sac lesions, factors similar to those of *MG* or *MS* infections may be involved. Ascites formation in turkeys is suggested to be a manifestation of liver damage. Osteodystrophy seems to be caused by interference
with the amino acid metabolism in the tissue. *MM* is synergistic with *MI* and *MS* in producing airsacculitis (80).

**Clinical signs**

Young turkeys are more sensitive than older birds. Reduction in growth rate, skeletal abnormality (perosis, twisting and shortening of the tarso-metatarsal bone, hock joint swelling and crooked necks), abnormality in the primary wing feathers, sternal bursitis, sinusitis and decreased hatchability are characteristic (43, 94).

**Lesions**

Pathological characteristics that should be mentioned are as follows: thickened air sac wall, caseous exudate in the thoracic and abdominal air sacs, skeletal abnormalities (such as perosis affecting the tibiotarsal and tarso-metatarsal bones and lateral deviation of the cervical vertebral column), synovitis and ascites.

**Diagnosis**

Clinical signs, lesions and poor hatchability suggest *MM* infection. However, infection may occur without signs and lesions. Therefore, diagnosis should be made in the laboratory following the same guidelines as for *MG* and *MS*. A peculiarity is that *MM* can easily be isolated from the air sac, phallus, cloaca, trachea and sinus. The medium required is similar to that for *MG*. To reduce contamination, polymyxin B (100 units/ml) and Mycostatin (50 units/ml) are recommended, in addition to penicillin and thallium acetate. In biochemical examination, hydrolysis of arginine, phosphatase activity and inability to ferment glucose should be considered. SPA is less sensitive. It begins to show positive results at an age of three to five weeks. More reactors can be detected in flocks with active air sac lesions than in the case of phallus infection. Vaccination with *Erysipelothrix* vaccine may induce non-specific SPA. HI is not reliable, since haemagglutination activity of strains is quickly lost. No antigen is commercially available and it is difficult to prepare a satisfactory HI antigen. Indirect ELISA can be used as a confirmatory test. DNA probes and PCR techniques are available (106).

**MYCOPLASMA IOWAE INFECTION**

*Mycoplasma iowae* (*MI*) is generally associated with embryo mortality, moderate airsacculitis and leg abnormalities in chickens and turkeys, and with reduced hatchability in turkeys. *MI* was isolated predominantly from turkeys and is currently becoming an important poultry pathogen (17, 18, 56).

**Epizootiology**

*MI* infection occurs in North America and western and eastern Europe (56). It has been isolated from turkeys and chickens. The spread of infection has not yet been studied thoroughly. Transmission through the egg and veneral spread are known in turkeys.
Aetiology

MI catabolises both glucose and arginine and reduces tetrazolium but does not produce phosphatase or film and spots. It grows in the presence of 0.5% to 1% bile salt. Strains of this species have blebs, like the attachment organelle of MG (70). The MI species contains six different serovars (I, J, K, N, Q and R) (43, 44). Marked strain differences were observed concerning pathogenesis, serological properties and proclivity for niche (50, 70). A relatively high homologous pattern in Coomassie blue-stained SDS-PAGE gels can be seen with only minor variations in the molecular weight range of 29 to 45 kDa and 116 to 205 kDa. However, marked variations were observed among MI strains using Western blotting, in the distribution of MAb-recognising antigens. This also complicates the detection of serum antibodies in experimentally or naturally infected birds. It is interesting to note that strain PPAV, belonging to this species, was isolated from the apple seeds of trees affected by proliferation disease and showed minimal differences (90% of DNA-DNA homology; only five different bases in total 16S rRNA) in comparison with strain 695, but was more related to strain 693 of the serotype J (37). Therefore, it was suggested that the apple from which PPAV was isolated had been contaminated with MI by birds. Strains of this species are very resistant to various conditions as they can survive in the intestinal tract of the host.

Clinical signs

A variety of disease manifestations, including high embryo mortality, mild airsacculitis and leg and joint abnormalities have been described as characteristic of natural and experimental infection with MI (17, 19). Reduction of hatchability in a turkey flock can reach 2% to 5%.

Lesions

In embryos, stunting, congestion, hepatitis, oedema and splenomegaly are observed. In chickens and turkeys, lesions include poor feathering, tenosynovitis, leg abnormalities (i.e. chondrodystrophy, rotated tibia, toe deviation or rupture of the digital flexor tendon), bursal atrophy and moderate airsacculitis.

Diagnosis

High embryo mortality is indicative. Diagnosis can be made by isolation and identification of MI strains. MS can be isolated from the gastrointestinal tract, cloaca, oviduct and phallus. The identification should follow the same steps as described for MG identification. An important biochemical characteristic of MI is the ability to ferment glucose and hydrolyse arginine. Serology is not reliable. Recently, a species-specific DNA probe (104) and PCR (50, 107), as well as multi-species PCR, were developed. In the latter case, the PCR product was differentiated by RFLP studies using restriction enzymes Hpa I, Hpa II and Mbo I (34).

MYCOPLASMA ANSERIS INFECTION

Mycoplasma anseris (MA) was described by Bradbury et al. (19). Infections occur in geese, resulting in airsacculitis, peritonitis and embryo mortality. Mycoplasma
infection occurs mainly in European countries where geese are raised; however, the incidence of the disease varies greatly, depending on the size and density of the flock and hygiene conditions.

**Epizootiology**

*MA* has been detected in Hungary, the Czech Republic, France and China. Infection spreads within a flock or between flocks by direct contact of infected birds with non-infected geese. Transmission of *MA* through the egg has been proved. *MA* can be isolated from the air sacs, peritoneum, oviduct, testis, phallus lymph and embryos. The incidence of isolation is relatively low (88).

**Aetiology**

Relatively few strains were studied and no differences in biochemical or serological characteristics or antibiotic sensitivity were found. Infection is often associated with parvovirus and *E. coli* infection, causing more severe disease.

**Clinical signs**

Infected birds do not show any characteristic signs. Slight nasal discharge, retarded growth rate (15% to 20%), decreased body weight and a 1% to 2% increased mortality can be noticed in two-to-six-week-old goslings. Sometimes temporary lameness occurs. If geese are force fed and kept in a closed building, definite signs of respiratory symptoms (i.e. nasal discharge, sneezing, coughing, difficult breathing and 5% to 10% mortality) can be noticed in the second and third weeks of force feeding. In layers, the first two months of egg production are normal. However, in the third and fourth months of egg production, a significant decrease in the number of eggs laid can be observed. Affected flocks stop egg production earlier than non-affected flocks. Infertility or embryo mortality increases by up to 10% to 40%. The body weight of goslings hatched from eggs of infected flocks is considerably lower than that of goslings from healthy flocks. Offspring gosling flocks from infected layers are generally retarded in growth (97).

**Lesions**

Lesions include a cloudy, thickened air sac wall, accumulation of serous-fibrinous exudate in the air sacs and peritoneum and swollen joints in young goslings. In geese which die during force feeding, thickening of the air sac wall and an accumulation of caseous masses in the air sacs and peritoneum are significant.

**Diagnosis**

*MA* infection can be confirmed only by isolation and identification of the agent. In this work, a similar procedure to that described for *MG* should be followed. No reliable serological test is available. However, indirect haemagglutination can detect antibodies to *MA*.

**MYCOPLASMA SPECIES 1220 INFECTION**

The incidence of *Mycoplasma* sp. (*M. sp.*) 1220 infection is high in geese. Its presence is usually connected with the same types of disease as *MA* infection, but
M. sp. 1220 is also associated with inflammation of the phallus in ganders, causing very significant economic losses to goose production (89).

**Epizootiology**

This micro-organism can easily be cultured from the air sacs, peritoneum, oviduct, testicles and other parenchymatous organs, as well as from dead embryos. An interesting feature of this organism is that it can be isolated very often from phallus lymph of ganders. If the ganders are sexually inactive, the isolation rate is 10% to 20%. However, at the beginning of sexual activity, before starting egg production, the incidence can reach 60% to 70% and 90% at the peak egg production period (89). If this organism is present, phallus inflammation frequently develops. Massive occurrence of the disease can be observed in flocks where bird density is high, space and water supply are limited and where birds from different sources (especially young birds and old ones) are mixed. The disease occurs both in flocks with a normal egg production cycle and in flocks on the second cycle. At the beginning of egg production, 0 or 1% to 2% of clinically affected ganders can be found in flock. After one to two months of egg production, the incidence of affected ganders can reach 30% to 40%. During the entire egg production period, 50% to 70% of ganders can be diseased. If diseased ganders are replaced by healthy birds, within one month, approximately 30% to 50% of the new birds show clinical symptoms. In the second and third years, the disease can develop again. In such a flock, the ratio of males and females is reduced to 1:1 instead of 1:4. In flocks free from this organism, phallus inflammation has never been observed.

**Aetiology**

M. sp. 1220 strain seems to be biochemically and serologically unique. In comparative studies, M. sp. 1220 was definitely different from the reference strains of all known mycoplasma and acholeplasma species (89). However, some subclones of the original strain gave, in certain cases, a weak reaction in epifluorescence with M. anatis. Molecular biological studies are in progress to achieve a final identification. In artificial challenge experiments, after intravaginal infection of adult geese, M. sp. 1220 caused peritonitis in approximately 50% of animals and oviduct inflammation in layers. After inoculation of 10-day-old embryos, approximately 70% died with characteristic lymphoid tissue activation.

**Clinical signs**

The clinical picture of this disease in two-to-five-week-old goslings, geese in force feeding and layers is similar to infection described in MA. Phallus inflammation is characteristic. At the beginning of the disease, sexual activity of ganders is decreased and the mucosal membrane of the phallus becomes red, oedematous, and later necrotic. Finally, the phallus can slough. Embryo mortality or infertility is significant.

**Lesions**

In regard to M. sp. 1220 infection, similar lesions are seen as in MA-infected flocks. In addition, phallus inflammation or necrosis is present. A huge amount of fibrinous
mass accumulates around the base of the phallus. Airsacculitis, peritonitis and atrophying of the testicles are also common.

Diagnosis

Detection of the infection can be made based on isolation and identification of the micro-organism. The indirect haemagglutination test is indicative only.

**UREAPLASMA GALLORALE INFECTION**

*Ureaplasma gallorale* (UG) was first isolated from chickens and turkeys by Stipkovits *et al.* (86). The isolates from chickens and turkeys were serologically related to each other. The avian UG was completely different in one- and two-dimensional PAGE from all human and bovine strains and from several other ureaplasma strains (74). The occurrence of ureaplasmas in birds was confirmed by others (40, 61), and the organism subsequently received the name *Ureaplasma gallorale* (62). Very little is known about spread of infection and pathogenicity. Detection of UG infection in turkey flocks was associated with the problem of high (more than 60%) infertility (87) and a decrease in semen quality (a reduction of the number and motility of spermatozoa and an increase in the number of dead [30% to 50%] and abnormal [10% to 30% deformed, agglutinated] spermatozoa of toms). In some cases, artificial challenge of chickens produced no clinical signs or macroscopic lesions (61). However, in other cases, fibrinous airsacculitis and peritonitis, as well as a serological response, were induced by introducing UG into the nasal cavity, trachea and air sac (86). Alternatively, injection of UG into the infundibulum resulted in a significant reduction in egg production and in development of oviduct lesions.

**TREATMENT AND CONTROL OF MYCOPLASMA DISEASES OF POULTRY**

Mycoplasma diseases are economically important, egg-transmitted, hatchery-disseminated diseases. Therefore, therapeutic and prophylactic treatments, as well as control, are essential. The control procedure is composed of different measures, essentially concerning the improvement of hygiene, regular testing of flocks for the presence of mycoplasma infection with appropriate methods, application of proper antibiotics for treatment (for therapy or prophylactic medication or eradication), or vaccination. Since the treatment and control programmes for all mycoplasma species are essentially similar, a general description will be given.

**Treatment**

To achieve the required results from treatment, appropriate drugs should be selected. Mycoplasmas are resistant to antibiotics inhibiting cell wall synthesis (such as penicillins) or inhibiting membrane synthesis (e.g. polymyxin B). However, they are sensitive to antibiotics inhibiting protein synthesis. Many antibiotics such as tetracyclines (oxytetracycline, chlortetracycline, doxycycline), macrolides
(erythromycin, tylosin, spiramycin, lincomycin, kitasamycin), quinolones (imequil, norfloxacin, enrofloxacin, danofloxacin) or tiamulin (10, 60, 94, 98) have been reported as effective drugs. At the same time, studies reported variable results of treatment, reflecting the resistance of strains involved, different complicating infections, the wide spectrum of age groups and breeding lines, the time of experiments and conditions of hygiene. To overcome this problem, the following steps are recommended:

a) First, strains present in the flock should be tested for sensitivity to the antibiotic to be used. According to recent data, the lowest minimal inhibitory concentration (MIC) values are now produced by tiamulin, danofloxacin, enrofloxacin and lincomycin (45, 46, 60). The ordering of mycoplasma species according to antibiotic sensitivity from lowest MIC to highest MIC is as follows: MG, MS, MM and MI. However, there are big variations in antibiotic sensitivity among strains of one species. It is interesting to note that erythromycin is generally effective only against glucose-fermenting strains, while arginine-hydrolysing strains (i.e. MM and MI) are generally resistant.

b) As mycoplasmas are essentially localised in the mucosal membrane of the respiratory and reproductive tracts, it is recommended that antibiotics which accumulate in high concentrations in the tissues of these organs are selected. In this respect, tiamulin and enrofloxacin seem interesting, reaching 500 to 1,000 times higher concentrations in these tissues than their MICs against mycoplasmas.

c) Since mycoplasma infections (principally MG, MS and MM) are complicated with bacterial infections, it is recommended to select antibiotics which have, in addition to an antimycoplasma effect, an antibacterial effect, such as enrofloxacin or danofloxacin. Lincospectin offers a similar advantage, affecting both mycoplasmas and bacteria. Recent studies by the authors demonstrated a significant synergistic effect when using combinations of tiamulin with chlortetracycline or oxytetracycline, both against the mycoplasma and most respiratory bacterial pathogens, achieving a good therapeutic result.

Drugs can be administered by injection or in drinking water (for three to five days) or feed (for five to ten days). In general, therapeutic treatment has limitations, since severely diseased birds cannot be treated effectively. It should be remembered that treatment of infected birds will not result in the total elimination of infection. Therefore, the treatment should be repeated from time to time; generally once a month.

In flocks expected to be infected (i.e. originating from an infected parent flock or used in multi-age operations), it may be necessary to use the drugs metaphylactically or prophylactically. Prophylactic medication can be administered in the first week of life (to reduce infection originating from eggs), at the ages of three and five weeks (for broilers) or in expected stress periods (vaccination, regroupment, transportation and so on, for replacement pullets) to reduce the activation of infection. An essential step is that breeders should be medicated to reduce egg transmission of mycoplasmas.

Finally, it is essential strictly to respect the principles governing the use of antimicrobial agents, in order to avoid the emergence of resistant strains and to secure the safety of products of animal origin.
ERADICATION OF MYCOPLASMA INFECTION

The eradication of MG, MS and MM infections (no experiences are available for other mycoplasma species) consists of several steps, as follows:

**Improvement in hygiene and management conditions**

Hygiene conditions should be improved, as should management practices, such as methods of insemination or sexing in turkey flocks. Vaccination and prevention programmes against associating infections should be established to reduce the excretion of mycoplasmas into the environment.

**Treatment of breeder layers**

Breeder layers should be treated with proper antibiotics to reduce egg transmission of mycoplasmas.

**Treatment of eggs**

Eggs may be treated in the following various ways:

a) Injection of antibiotics such as lincomycin-spectinomycin (91), or gentamycin or tylosin (35) into the air cell of hatching eggs, or into the small end of turkey eggs. Edson et al. (26) have developed an equation based on the Poisson distribution to predict the chance of success of eradication of MM: \( p(o) = e^{-(a+b)h} \), where the probability of success \( (p[o]) \) was described by the number of eggs treated \( (n) \), the pre-treatment infection rate of the eggs \( (a) \), the treatment failure rate \( (b) \) and the hatchability of treated eggs \( (h) \). Decreasing the size of any of these four parameters (i.e. reducing the size of the flock to be treated, reducing the infection rate by medicating the flock or by selecting seronegative birds based on serological or cultural examination, or causing a decrease in treatment failure by using very effective antimycoplasma drugs) increases the likelihood of eradication. This procedure can be useful for MG, MS and MM infections but may depress hatchability by 10%.

b) Egg dipping (39). Eggs warmed to 37.8°C are immersed in cold antibiotic solution (tylosin or erythromycin) for 15-20 min to lower the incidence of egg-transmitted mycoplasmas. Penetration of antibiotic solution can be enhanced by pressure difference (i.e. the vacuum system) (2). The procedure is not favourable for hatching.

c) Egg heating. Room temperature eggs are heated during a 12-14 h period to reach an internal temperature of 46°C to kill mycoplasma in eggs (95). In this technique, hatchability may be reduced by 8% to 12% and it is not really effective for MM eradication.

None of these procedures can eliminate all mycoplasmas in all eggs. Therefore, mycoplasmas can pass through eggs to the progeny flock. For this reason, the progeny flock should be kept in small groups and should be controlled regularly for mycoplasma infection. Sera from at least 10% of the birds should be tested by serological methods, using HI or ELISA, and clinical samples should be tested by culturing or by the PCR technique, regularly. Positive groups should be eliminated as soon as possible and negative groups should be reared in strict isolation to avoid the introduction of infection. Establishing and maintaining pathogen mycoplasma-free
status of breeder flocks can be accomplished by participation in control programmes (e.g. the National Poultry Improvement Plan in the USA or the National Sanitary and Hygienic Control programme in France).

LES MYCOPLASMOSES AVIAIRES. – L. Stipkovits et I. Kempf.


LAS MICOPLASMOSIS EN AVES DE CORRAL. – L. Stipkovits e I. Kempf.

Resumen: Entre los más importantes micoplasmas aislados en especies aviares domésticas se cuentan Mycoplasma gallisepticum, M. synoviae, M. meleagridis y M. iowae. M. gallisepticum es el agente de la enfermedad respiratoria crónica en el pollo y de la sinusitis infecciosa en el pavo, dos enfermedades que causan substanciales pérdidas económicas. M. synoviae es responsable de sinovitis infecciosa o de enfermedades benignas de las vías respiratorias superiores. M. meleagridis infecta únicamente al pavo, en el cual provoca inflamación del saco aéreo, así como una disminución de los niveles de producción y de empollamiento. M. iowae está asociado a una caída del empollamiento en los criaderos de pavos. La transmisión se produce bien por vía directa, de individuo a individuo o a través del huevo, o bien de forma indirecta. La realización del diagnóstico se basa en el aislamiento e identificación de micoplasmas mediante pruebas bioquímicas, serológicas o moleculares, o bien en el estudio serológico del huésped por técnicas de
aglutinación en placa, de inhibición de la hemaglutinación o por ensayo
inmunoenzimático. Para un tratamiento terapéutico o profiláctico pueden
emplearse antibióticos (esto es, tetraciclinas, macrólidos, quinolonas y
tiamulina). La erradicación de las infecciones micoplas máticas requiere
prácticas de higiene y de manejo más cuidadosas, así como el tratamiento
terapéutico de las gallinas ponedoras y/o de los huevos por incubar y la mejora
de los procedimientos de monitoreo.

PALABRAS CLAVE: Aves de corral – Diagnóstico – Micoplasmas aviares –
Mycoplasma gallisepticum – Mycoplasma iowae – Mycoplasma meleagridis
– Mycoplasma synoviae – Síntomas.

* *

REFERENCES

detection of avian mycoplasmas in culture. Avian Dis., 39, 85-93.
sensitivity of two commercial enzyme-linked immunosorbent assay kits, the serum plate
agglutination test and the hemagglutination-inhibition test for antibodies formed in
polyacrylamide gel electrophoresis purified proteins of Mycoplasma gallisepticum and
M. synoviae as antigens in a dot-enzyme-linked immunosorbent assay. Avian Dis.,
34 (3), 575-584.
Mycoplasma gallisepticum and Mycoplasma synoviae studied by immunoblotting. Vet.
gallisepticum strains and identification of immunogenic integral membrane proteins
turkeys to strain S6 and variant Mycoplasma gallisepticum studied by immunoblotting. Avian. Dis., 36, 69-77.
and attachment to chick tracheal rings by antibodies to a 64-kilodalton membrane
(1989). – Identification of the antigenic components of the virulent Mycoplasma
gallisepticum (R) in chickens: their role in differentiation from the vaccine strain (F).


