Avian mycoplasmosis (*Mycoplasma gallisepticum*)

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Summary

*Mycoplasma gallisepticum* is the most economically significant mycoplasma pathogen of poultry, and has a world-wide distribution. In common with other mycoplasmas, *M. gallisepticum* is minute in size with minimal genetic information and with a total lack of a bacterial cell wall. These properties are reflected in a high degree of interdependence between *M. gallisepticum* and the host animal, and in the fastidious nature of the organism *in vitro*. Strains of *M. gallisepticum* differ markedly with respect to important biological properties such as pathogenicity, infectivity, tissue tropism and transmissibility. In addition, phenotypic variation of major surface antigens occurs at high frequency, which is a probable explanation for chronic infection by *M. gallisepticum* despite a strong immune response.

Infection with *M. gallisepticum* has a wide variety of clinical manifestations, but even in the absence of overt clinical signs, the economic impact may be significant. The most dramatic disease presentation of *M. gallisepticum* is chronic respiratory disease in meat-type birds, often as one of several aetiological agents in a multi-factorial disease complex. Transmission of *M. gallisepticum in ovo* from infected breeder birds to progeny is the major route of dissemination of the infection, and is the prime consideration for international trade.

In most countries, control programmes for *M. gallisepticum* are based on maintaining commercial breeding stock free of infection. In instances where control of *M. gallisepticum* infection is not feasible, vaccination, especially with newly developed live *M. gallisepticum* vaccines, is being evaluated as an option. Major advances in diagnostic methods have been made in recent years. Control programmes have been based on serological methods, with screening for infection usually accomplished by the slide plate agglutination (SPA) test or by enzyme-linked immunosorbent assay. Further serological testing and/or demonstration of the presence of the organism must be used to confirm SPA suspected positive tests. In principle, detection of the presence of the *M. gallisepticum* organism can be by isolation of the organism or detection of the deoxyribonucleic acid by molecular methods. Polymerase chain reaction represents a rapid and sensitive alternative to traditional culture methods, which require time-consuming specialised techniques. The development of molecular typing methods affords new opportunities for epidemiological studies and identification of reservoirs of infection.

Keywords

Avian diseases – Biosecurity – Chronic respiratory disease – In ovo transmission – Molecular typing – Mycoplasma gallisepticum – Vaccination.

Aetiological agent

The Mollicutes, more commonly known as mycoplasmas, are distinguished phenotypically from other bacteria by their minute size and total lack of a cell wall (122, 123). The presence of only a minimal amount of genetic information accounts for the complex nutritional requirements of these organisms, reflected in an obligate parasitic mode of life, with
a high degree of interdependence between the mycoplasma and the host animal. The fastidious nature of mycoplasmas is also reflected in the culture conditions required in vitro, with the consequence that isolation of the organism is usually performed in specialist laboratories (73). In the absence of a cell wall, organisms are highly pleomorphic and are not usually detectable by standard light microscopy of Gram-stained cultures or tissue smears. The typical 'fried egg' morphology of the microscopic colonies results from insinuation of the tiny pleomorphic organisms among the fibres of the mycoplasma agar medium where growth is initiated. Further division of cells, as the colony develops, results in spread onto the surface of the agar (120). In addition, since mycoplasmas are not sensitive to antibiotics that affect cell wall synthesis, these antibiotics may be incorporated in culture media used for isolation of mycoplasmas, in order to inhibit growth of other bacteria present in clinical samples (69, 73).

Most species of mycoplasma are parasites of animals or plants, including more than a dozen species which are known to infect chickens or turkeys (67). Of these, Mycoplasma gallisepticum and M. synoviae are pathogenic for chickens and turkeys, while M. iowae affects primarily turkeys, and M. meleagridis only infects turkeys. Mycoplasmas tend to be highly host-specific, although improved diagnostic methods and precise molecular methods for species identification may necessitate some re-evaluation of earlier views (6, 137). Avian mycoplasmas are not known to infect humans or other mammalian species, although isolations from domestic animals have been reported sporadically. Mycoplasma gallisepticum may infect many species of birds, but gallinaceous birds are most susceptible.

The primary habitats of mycoplasmas are the mucosal membranes of the respiratory tract, and/or the urogenital tract, eyes, mammary glands and joints. Most mycoplasmas are considered surface parasites, rarely invading tissues, although spread to other organs strongly suggests a transitory systemic infection, at the least. Adhesion of mycoplasmas to host cells is a prerequisite for successful colonisation, and ensuing pathogenesis (76, 121). New information, relating principally to mycoplasmas infecting humans, suggests that some species may penetrate cells and survive intracellularly (123). In general, mycoplasma species, and possibly also specific strains, exhibit organ and tissue proclivity, although the organism is not necessarily completely excluded from other organs. Mycoplasma gallisepticum is one of the species of mycoplasma that, as primary pathogens, can cause acute and chronic diseases at multiple sites, with wide-ranging complications.

Mycoplasmas, including M. gallisepticum, have recently been demonstrated to have the ability to vary the expression of major surface antigens, thus expressing a continually changing 'antigenic profile' to the immune system (123). Variability occurs not only among strains, but within clones of a single strain (10, 40). Continual variability in the expression of such surface antigens also occurs in vivo (87) and may be a major factor in the development of clinical disease in addition to having a significant impact on the development of serological responses (96). The marked heterogeneity with respect to presentation of the major surface antigens provides a likely explanation of how mycoplasma infections are able to persist in birds despite a strong immune response.

Resistance to the clinical manifestations of mycoplasmal infection, and positive response to antibiotic therapy for disease require an intact immune system (6, 18). In humans, mycoplasmas are being increasingly implicated as co-factors in new diseases or in diseases of previously unknown aetiology. This has been attributed to improvements in diagnostic techniques and an increase in the susceptible population of immunodeficient patients (due to human immunodeficiency virus, cancer chemotherapy, and others) (6). At present, little is known about the pathogenesis of mycoplasmas in immunocompromised poultry. However, unusual disease syndromes are often present in flocks that are immunocompromised because of infectious or non-infectious agents (129). Complicated infections of multiple aetiologies, probably including one or more avian mycoplasma species, are frequently present under commercial conditions and present a challenge to diagnosis and treatment (68, 69).

Description of the disease

The most economically significant mycoplasmal pathogen of poultry is M. gallisepticum (74, 94). The major consideration for international trade is the necessity and the ability to determine the M. gallisepticum status of the imported material (most often hatching eggs or one-day-old chicks). In most cases, the M. gallisepticum status of the progeny chicks is determined by the breeder flock from which they are derived. In this section, some of the factors which affect M. gallisepticum infection of embryos and chicks and the ability to detect this infection will be discussed. The clinical manifestations of M. gallisepticum infection will also be discussed, since an understanding of the disease is necessary to evaluate policy.

Infection with M. gallisepticum may have a wide diversity of clinical manifestations, of which chronic respiratory disease (CRD) and downgrading of carcasses in meat-type birds are probably the most dramatic (68, 94). Descriptions of gross lesions and histopathology may be found in a recent review and the references therein (94). Mycoplasma gallisepticum infection alone may cause respiratory disease in turkeys, but is most often mild or sub-clinical in chickens. However, M. gallisepticum is frequently present as one of several aetiological agents in a multi-factorial disease complex (53, 68). The interactions among M. gallisepticum, various respiratory viruses, Escherichia coli, Haemophilus
paragallinarum, and others have been documented (49, 68, 107). Vaccine strains of Newcastle disease virus or infectious bronchitis virus may give marked respiratory reactions in M. gallisepticum-infected birds, necessitating the use of milder vaccine strains under these conditions (94, 106). The route of exposure and the infectious dose of the microbe, in addition to environmental and stress factors, such as temperature and ammonia concentration, age and type of birds, are among the factors that influence mycoplasmosis (94). Moreover, infection with M. gallisepticum may cause a marked reduction in feed consumption efficacy with a significant economic impact, even in the absence of overt clinical signs.

Loss of production in laying birds may also occur as a result of M. gallisepticum infection, and is particularly marked in table egg layers infected at peak lay (44). Early infection of pullets with M. gallisepticum can protect the birds against the pathogenic effects of later infection (34). This is the principle underlying the successful use of live M. gallisepticum vaccines in layer flocks, as is widely practised in the multi-age layer operations in the United States of America (USA) and to a limited extent elsewhere (19, 139). Mycoplasma gallisepticum has been implicated in salpingitis and other pathologies of the reproductive system, although whether this is the principal or sole cause of reduction in egg production is not clear (30, 110).

In common with all the pathogenic avian mycoplasmas, M. gallisepticum is transmitted in ovo. Infection of the embryo by M. gallisepticum and transmission to the progeny (egg transmission) probably occur as a sequel to acute respiratory infection, due to contiguity of the abdominal air sacs to the oviduct (125). Very little information is available about rates of transmission, and these rates are likely to vary widely under different conditions, among individual birds, and at different times during the same outbreak. The highest frequency of transmission is found during the acute phase of the disease when M. gallisepticum levels in the respiratory tract reach a peak (44, 96). In separate studies of experimental reproduction of the disease in breeder hens, peak transmission to eggs after respiratory challenge by M. gallisepticum was detected at four weeks post infection in approximately 25% of the progeny (45) and in over 50% at three to six weeks (130). At eight to fifteen weeks after infection, transmission levels were approximately 3% (125) or approximately 5% at twenty to twenty-five weeks post infection (45). However, some individual birds showed consistently high transmission rates throughout the test period. During chronic infection under field conditions, transmission to eggs is likely to occur at much lower levels. Transmission of M. gallisepticum from infected chicks at hatch is a major source of infection whereby even low levels of transmission to the egg result in widespread infection among the progeny. However, at low transmission rates, detection of the infection by sampling embryos or one-day-old chicks requires a very large sample size to be statistically feasible.

Embryo mortality, which may occur as a result of infection with M. gallisepticum, has been shown in experimental infection studies to be affected by strain differences (83, 118). However, the pathogenicity in ovo does not correlate with the pathogenicity of the M. gallisepticum strain in respiratory infection of the fowl in vivo (84). The presence of maternal antibodies to M. gallisepticum in the embryonated egg has been found to reduce the in ovo pathogenicity of infection, increasing the probability of survival of the infected embryo (83). However, M. gallisepticum infection may weaken the embryo, resulting in difficulty in hatching (pipped embryos) or low-quality chicks.

With the exception of in ovo infection, the upper respiratory tract is generally accepted to be the portal of entry in natural infection by M. gallisepticum. The trachea also appears to be the preferential site of infection for most strains of M. gallisepticum, although disease signs are usually manifested in other parts of the respiratory system (air sacs or sinuses). The trachea serves as a reservoir of infection and is also readily accessible for sampling the live bird. Levels of M. gallisepticum in the trachea are highest at the acute stage of infection, often before the first serological response is detected in all the birds (85, 144). Detection by culture or other methods for direct detection of the organism is most successful at this stage of infection (52). Levels of M. gallisepticum in the trachea decrease from $10^7$ to $10^2$ colour changing units with progression of the infection. In the absence of exacerbating environmental stress or infection with other pathogens, the progressive infection in the trachea may be self-limiting (144), but tracheal infection persists in the presence of humoral or local antibody (66, 144). Tracheal levels in chronically infected birds, such as commercial egg layers several months after infection, may be so low that M. gallisepticum is not detected by usual sampling and culture methods. Antibiotic treatment may also markedly reduce tracheal levels, although non-viable cells may be present, which can be detected by molecular methods (26, 58).

Although intercurrent infections and environmental factors influence the outcome of M. gallisepticum infection, marked heterogeneity has been observed within the species with respect to important biological properties. Variability in tissue tropism is indicated by isolation of an encephalitic form of M. gallisepticum from turkeys (22, 136). An M. gallisepticum variant with proclivity for the cloaca produced a persistent low level serological response (102). A strain of M. gallisepticum with proclivity for the eye, producing unilateral enlargement of the eyeball, has been reported (119), in addition to a natural outbreak of keratoconjunctivitis in layer chickens, apparently caused by M. gallisepticum (109). Mycoplasma gallisepticum has been demonstrated to be the aetiological agent of conjunctivitis in free-ranging house finches (Carpodacus mexicanus) and American goldfinches (Carduelis tristis) in the eastern USA and Canada (91, 99). The M. gallisepticum isolates from song
birds comprise a unique biotype according to biological properties and molecular typing (92).

So-called variant strains, characteristically differing from prototype M. gallisepticum by reduced virulence, transmissibility and antigenicity, were first described in the early 1970s (150). More recently, similar strains were recognised in turkeys (5). In experimental infection studies, variant strains of M. gallisepticum were often difficult to reisolate from individual birds, but persisted in the flock and were particularly problematic with respect to diagnosis (29, 62). Although variant strains were originally regarded as exceptional and unusual phenomena, these now appear to be extreme and stable examples of a spectrum of variable biological and antigenic properties within the M. gallisepticum species. Important examples of 'atypical' M. gallisepticum strains are the live vaccine strains F, ts-11 and 6/685 which will be discussed in detail at the end of this paper. Each of the vaccine strains differs markedly from the others and from fully pathogenic M. gallisepticum, in biological properties such as infectivity, transmissibility, persistence and immune response, in addition to genetic and antigenic markers which can be detected in molecular diagnostic tests.

Diagnostic methods

Whatever method is employed for mycoplasma control, rapid and precise diagnostic methods are of paramount importance. Major advances in diagnostic methods have been made in recent years, and when validated by field experience, are reflected in the periodic updates of standard procedures. Information may be found in publications supporting the National Poultry Improvement Plan (NPIP) (3) and in the Manual of Standards for Diagnostic Tests and Vaccines of the Office International des Epizooties (OIE) (73) as well as in peer-reviewed publications that relate to specific methods.

The basis for control programmes has centred on serological methods, both screening and confirmatory, with reagents confirmed by isolation of the organism. In international trade, the M. gallisepticum status of chicks (or embryos, in the case of fertile eggs), is usually determined by the testing and certification of the breeder flock of origin. Diagnosis is made on a flock basis, and the presence of one or more infected birds in the flock sample constitutes an infected flock. Factors which affect the accuracy of the testing procedures are the sample size, the time period between tests, and the timing of the test with respect to shipment of the chicks. General guidelines of the NPIP require testing of 10% of the flock (a minimum of 300 birds) before the onset of egg production and every sixty to ninety days thereafter (3). Regulations and policy instituted by breeder companies relating to flocks from which fertile eggs are exported are often more stringent, mandating serological screening at more frequent intervals (for instance, every two weeks) in order to obtain test results while the eggs are still in the hatchery. As mentioned above, egg transmission occurs at the highest levels during the early stages of infection, and this is the critical period with respect to the status of the exported material. Negative serological results in the breeder flock prior to shipment of the progeny are a good indication that the flock was free of M. gallisepticum when the eggs were set in the hatchery twenty-one days earlier.

Diagnosis of M. gallisepticum infections in poultry breeder flocks is often performed in the absence of overt clinical signs, and screening for infection is usually accomplished by the slide plate agglutination (SPA) test with commercial stained antigen. Slide plate agglutination is highly efficient in detecting immunoglobulins of the immunoglobulin M (IgM) class, which are the earliest response to mycoplasma infection (64). Although the test is rapid, highly sensitive and relatively inexpensive, care must be taken to perform the test according to the instructions of the antigen manufacturer, including appropriate control sera (73). Sets of positive control sera from chickens infected by contact with M. gallisepticum or M. synoviae and negative control sera are currently available from the Poultry Diagnostic Research Center, University of Georgia, Athens, USA. The greatest disadvantage of the SPA test is low specificity, with false positive reactions and cross-reactions encountered relatively frequently. Serological detection of M. gallisepticum may be complicated by co-infection of flocks with M. synoviae, due to serological cross-reactions between the two mycoplasma species (4). In addition, non-specific serological reactions are frequently detected after use of inactivated vaccines (43). Further serological testing and/or demonstration of the presence of the organism must be used to confirm positive or suspected positive SPA tests. In some cases, marking of birds which are sampled may be desirable to facilitate repeat testing of those which give suspicious serological reactions.

Traditionally, the test of choice for confirmatory serology has been haemagglutination-inhibition (HI), which can be performed with fresh culture of a haemagglutinating test strain of M. gallisepticum (73) or with standardised preserved antigen (3). Diagnostically significant titres in the HI test may not be detected until three or more weeks after infection. However, the test is highly specific, even to the level of differentiation among strains (70). The major factors in support of alternative methods are the delay in development of HI antibodies, the strain specificity which may result in lack of detection of variant M. gallisepticum strains and the technical problems which may be encountered in producing high titre specific HI antigen and in performing the test.

Commercial enzyme-linked immunosorbent assay (ELISA) kits are widely available and are increasingly used for serological confirmation (59). Marked differences may be found among the different manufacturers, and care should be taken to use a product which has been validated with a wide
Spectrum of field samples and strains. The recommended ELISA kits have excellent sensitivity and specificity, but transitory non-specific reactions may still occur, for similar reasons to those occurring in the SPA test (4, 43). Potential improvements in ELISA specificity may result from the use of a blocking ELISA utilising an M. gallisepticum-specific monoclonal antibody (28), utilisation of highly purified antigens (103) or recombinant antigen, as suggested for M. synoviae ELISA (108). However, increasing the specificity presents the risk of decreasing the ability to detect all M. gallisepticum strains.

Some possible pitfalls exist in the dependence on serological testing for determination of flock status for M. gallisepticum. Temporal development of antibodies has been described in experimentally infected poultry (4, 64) and is presumed to follow a similar course in field infection. However, some flock treatments, such as the use of certain antibiotics, may affect the development of the immune response. In experimental infection trials, fewer serological responses were found in M. gallisepticum-infected chickens or turkeys treated with antibiotics than in the M. gallisepticum-infected non-medicated groups (54, 55, 56). Immune suppressive agents, infective or non-infective, may delay the onset of a detectable serological response to infection (129). Several studies have reported problems in detecting M. synoviae infection using the SPA test, primarily in turkeys (113). Although it is not clear to what extent, if at all, a similar phenomenon occurs with M. gallisepticum (114), some laboratories have introduced the ELISA test as a supplementary or alternative screening method. An ELISA kit which detects both M. gallisepticum and M. synoviae antibodies in a single reaction may be used for preliminary screening.

Serological testing of progeny chicks to determine M. gallisepticum status is sometimes desired, particularly when access to the parental flock is limited or when questions arise about the testing methods used at the place of origin. Immunoglobulin G passes from the maternal circulation into the yolk of the egg, with the pattern of rise and fall of antibodies following that of the serum, but delayed by five to six days, the time required for the maturation of the egg (48, 128). Transferred IgM has also been detected at low levels in the yolk, and more significant levels in the egg white (145). However, only maternal IgG, derived from the egg yolk, is present in the circulation of the chick (128). This passes from the yolk to the embryo during the last five or six days of embryo development and to the chick for approximately two days post hatching, at which times serum levels should peak. Catabolism of maternal antibody occurs during the first two weeks post hatching, with declining antibody titres. Synthesis of new antibodies in the chick, both IgG and IgM, begins in the first week of life, and is expected to reach adult levels at four to six weeks of age (81, 128). The ELISA is usually recommended for testing of yolk samples in fertile or non-fertile eggs, and testing of maternal antibodies in the chick (17). Reactions to the SPA test may also be present (131), but results should be treated with caution due to the possibility of non-specific reactions and the relatively low sensitivity of the test for IgG. Antibodies to M. gallisepticum have been detected in chicken bile by various serological tests, including the indirect immunoperoxidase assay, which may also be an option for progeny chicks (9).

In principle, the presence of the M. gallisepticum organism can be confirmed by isolation in mycoplasma media or by detection of the specific deoxyribonucleic acid (DNA). Isolation is still considered the 'gold standard', but the existence of circumstances where M. gallisepticum may be present but cannot be isolated even by the most skilful techniques, is now fairly well accepted. Detailed methods for culture and identification of M. gallisepticum may be found in the OIE Manual and other texts (69, 73, 74). The ability of culture media to support the growth of M. gallisepticum should be confirmed by testing with a low passage isolate. Identification of M. gallisepticum and differentiation from other mycoplasma isolates is usually based on immunological methods, most frequently immunofluorescence, requiring specific antisera that are not available commercially. An alternative method for identification is the use of DNA-based tests, using specific or universal mycoplasma tests (78, 80).

Polymerase chain reaction (PCR) represents a rapid and sensitive alternative to traditional culture methods which require specialised media and reagents and are time-consuming (57). A major advantage of the implementation of M. gallisepticum-PCR technology is that the investment in training and equipment can be exploited for diagnosis of an increasingly wide range of poultry diseases for which PCR is now one of the tests of choice.

Results of the PCR test can be obtained in one or two days, as opposed to the usual one to three weeks for isolation and identification of M. gallisepticum. Equally important is the ability to obtain accurate PCR results in the presence of mixed infection with several species of mycoplasma, contamination by secondary bacterial infections, and inhibition of growth by antibiotics, antibodies or other host factors. In particular, the problem of co-infection with saprophytic mycoplasmas that grow more rapidly than M. gallisepticum in enrichment cultures is a major impediment to isolation. Detection of DNA from non-viable organisms, for instance after antibiotic treatment, is a possible drawback to the PCR method (58).

The availability of a commercial kit for M. gallisepticum-PCR was a major impetus to the introduction and acceptance of the PCR technology as a supplementary diagnostic method for M. gallisepticum. An additional advantage of the commercial M. gallisepticum-PCR kit is the ability to differentiate between standard strains of M. gallisepticum and the F vaccine strain. Recently, increased interest has been shown in alternative, non-commercial PCR tests that are less expensive and somewhat more rapid than the commercial kit (78). All PCR
methods require specialised and precise technical skills in a dedicated laboratory. Due to the high sensitivity of the test, care must be taken to avoid false positive reactions due to extraneous DNA, but this can be prevented or detected by appropriate controls. A critical control in the use of PCR for diagnosis is the inclusion of an internal control to avoid ‘false negative’ results due to the presence of inhibitory substances in the reaction mixture. Amplification of the internal control amplicon, which can be readily differentiated from the target DNA amplicon, indicates that there are no inhibitors of the PCR reaction. Recently, an intrinsic control was developed for an M. meleagrisis PCR test (104), but such a control is not in routine use for M. gallisepticum-PCR testing.

A recent innovation in diagnosis is the development of molecular typing methods for differentiation of M. gallisepticum strains. The most commonly used molecular typing method is random amplification of polymorphic DNA (RAPD), a PCR-based technique which gives a unique strain fingerprint (35, 41). This technique is in routine use in a few specialist laboratories, and readily distinguishes among the live M. gallisepticum vaccine strains and the field strains present in natural infection (21, 75). The RAPD technique has also been used for molecular tracking of spread of infection among flocks and from putative reservoirs of infection in commercial poultry (20, 68, 89). The technique requires a high degree of technical expertise, and gives a satisfactory degree of reliability and reproducibility within each laboratory, but only to a limited degree between different laboratories. Thus, the precision necessary for construction of a database of strain-specific genomic fingerprints has not yet been achieved. A major impediment to widespread application is the necessity to perform RAPD on pure cultures of M. gallisepticum isolates. This requires specialised skills in addition to lengthening the time required to obtain results. Current research is attempting to develop rapid molecular methods for specific detection of M. gallisepticum biotypes, such as the live vaccine strains, as has been successful for the F vaccine strain (63).

Sampling, sample transport and processing for M. gallisepticum testing are highly critical stages in diagnosis. Sampling for M. gallisepticum in live birds is usually from the trachea or choanal cleft. In a comparative study, isolation rates were higher from the latter site, with less stress for the bird (16). During the acute phase of infection, between twenty and thirty individual samples for isolation are usually sufficient, whereas a larger sample size may be necessary at the chronic stage of infection. Swabbing technique, including the type of swab used and prewetting before sampling, may affect the success of isolation, especially when relatively few organisms are present (152, 153). Isolation of M. gallisepticum has been successfully performed from the cloaca in experimentally infected chickens, although this may be attributable to an unusual tissue proclivity in the M. gallisepticum strain used (102).

In birds sacrificed for sampling or in fresh carcasses, after necropsy, or from dead-in-shell or pipped embryos, isolation of M. gallisepticum may be performed successfully from a variety of organs, usually from the respiratory or reproductive tract (2, 73, 94). Isolation has been successful from the brain or the eye of fowl with relevant clinical signs (22, 109), in addition to bile of infected birds (9). Sampling of carcasses for isolation, including those that have been frozen, may be problematic due to the presence of bacterial contamination or lysis of cells which liberates inhibitory substances. Sampling for PCR must also assure that conditions are such that no degradation of the DNA occurs by intrinsic or environmental factors.

Several rapid methods for extraction of DNA from mycoplasma cells have been used for sample preparation for M. gallisepticum-PCR (58, 78, 79). Samples for PCR are often pooled (three to five tracheal swabs per PCR reaction) to increase sample size and reduce the cost of testing. However, pooling of samples may increase the possibility of inhibition by substances that may be present in the mucus or other tissue fluids, thus decreasing the sensitivity of the PCR test. When pooling large numbers of samples, purification of the DNA may be necessary using standard methods or rapid commercial kits.

Testing for the presence of M. gallisepticum in the embryonated eggs or progeny chicks by culture is not recommended as a routine method for determining the status of the flock. Low levels of in ovo transmission necessitate the sampling of a large number of embryos. Mycoplasma gallisepticum can be isolated with relatively high frequency from pipped eggs from infected flocks (77), and preliminary results suggest that this may also be a recommended sample site for PCR (A. Ramirez and S. Levisohn, unpublished findings).

Epidemiology

Mycoplasma gallisepticum infection occurs naturally in chickens and turkeys. Transmission of M. gallisepticum infection occurs by the following two major routes:

- vertically (in ova), from an infected breeder flock to the progeny
- horizontally, by direct or indirect contact of susceptible birds with infected carriers or contaminated debris.

Important characteristics of M. gallisepticum that affect the epidemiology of infection are the relatively stringent host specificity and the frequent occurrence of asymptomatic infection. Molecular diagnostic methods for highly sensitive and specific detection of M. gallisepticum have been developed recently, and these are important tools for studying the epidemiology of M. gallisepticum infection. Mycoplasma
Mycoplasma gallisepticum can now be detected with greater efficiency, in a wide variety of clinical samples. In addition, molecular typing methods for *M. gallisepticum* strains allow identification of specific strains of *M. gallisepticum*, as discussed in the section on diagnosis.

In addition to domestic poultry, *M. gallisepticum* has been reported fairly frequently in other gallinaceous birds, either free-ranging or maintained in captivity, and sporadically in birds of other genera (94). Natural infection with *M. gallisepticum* has been reported in a variety of game birds, in some cases with marked clinical signs. However, evaluation of the extent of this phenomenon is difficult, due to difficulties in isolating *M. gallisepticum* in the presence of high levels of saprophytic mycoplasmas (24, 94). In some cases, the source of infection appears to be *M. gallisepticum* present in nearby domestic poultry. Retrospective testing by *M. gallisepticum*-PCR of stored clinical samples from partridges and pheasants in the United Kingdom with upper respiratory disease demonstrated evidence for a higher rate of infection than was found by isolation (15). This supported the theory that *M. gallisepticum* is a major cause of disease in these birds, which also serve as a potential source of infection for domestic poultry. Experimental infection of red-legged partridges (*Alectoris rufa*) with *M. gallisepticum* demonstrated the occurrence of pathogenic effects and persistent carriage in this host (37).

*Mycoplasma gallisepticum* has been implicated as the causative agent in an outbreak of conjunctivitis in free-ranging house finches and American goldfinches, and in a case of conjunctivitis in a blue jay (*Cyanocitta cristata*) that was housed in a cage previously occupied by afflicted house finches (91, 99). Initially observed in 1994, the infection is now nearly ubiquitous in wild finches over the entire eastern range of these birds in the USA and Canada (36, 100). To investigate the molecular epidemiology of this outbreak, DNA fingerprints of isolates were made by RAPD. *Mycoplasma gallisepticum* isolates from songbirds (representatives of all three host species) examined from 1994 to 1996 in eleven States were compared with vaccine and reference strains and with contemporary isolates from commercial poultry. All *M. gallisepticum* isolates from songbirds had RAPD banding patterns identical to each other but different from other strains and isolates tested. The unique finch RAPD pattern is consistently found in all isolates from these birds (36, 92). These results indicate that isolates of *M. gallisepticum* from conjunctivitis in songbirds are all of the same molecular type, which suggests a single source. The RAPD typing results gave no indication that infection is shared between songbirds and commercial poultry. A slow spread to chickens has been observed under experimental conditions, but only mild clinical signs are detected (111, 132) and no natural infection of the unique finch biotype has been detected in domestic poultry (92).

Isolation of *M. gallisepticum* from the respiratory tract of ducks has been reported, with no apparent clinical signs (7). *Mycoplasma gallisepticum* was also isolated from embryonated duck eggs from the infected flock. The apparent source of infection was infected chickens which were raised together with the ducks. Experimental infection of specific-pathogen-free (SPF) ducks with *M. gallisepticum* resulted in colonisation but only limited respiratory signs (60). *Mycoplasma gallisepticum* and *M. synoviae* also were isolated from geese, apparently infected by contact with infected chickens, and from dead-in-shell goose embryos from the infected flock (8). Recently, *M. gallisepticum* was isolated from the air sac in fattening goose with typical signs of CRD (S. Levisohn, unpublished findings). Molecular typing of the *M. gallisepticum* isolates from geese gave the same RAPD pattern as that found in *M. gallisepticum* isolates from an outbreak in a broiler-breeder flock in the same region, although the geese and the chickens were not in contact at any time.

In examining clinical samples, care must be taken to differentiate between *M. gallisepticum* and the closely related species *M. imitans*, which is relatively frequently isolated from ducks and geese (12). Some early reports of *M. gallisepticum*, prior to recognition of the *M. imitans* species, may have been misidentified. A useful tool for differentiation of *M. gallisepticum* and *M. imitans* is an *M. gallisepticum*-PCR restriction fragment length polymorphism test which exploits a unique sequence difference in the 16S ribosomal ribonucleic acid (rRNA) genes of the two species (57).

The clinical significance of *M. imitans* in chickens and turkeys has not yet been established, although a potential for pathogenicity has been demonstrated in experimental infection studies. Mixed infection of *M. imitans* with infectious bronchitis virus in chickens (38) or rhinotracheitis virus in turkey poults (39) indicates a synergism between these agents. Experimental infection of SPF ducks with *M. imitans* resulted in colonisation of the respiratory tract, but no respiratory signs (61). *Mycoplasma imitans* was isolated only sporadically from experimentally infected SPF chickens, and no pathogenic effects were reported. In experimental infection of partridges, colonisation by *M. imitans* was lower and pathogenicity less than that found for *M. gallisepticum* (37).

Horizontal infection of *M. gallisepticum* occurs readily by contact with infected birds, most probably by the airborne route with organisms excreted from the respiratory system of infected birds. Experimental infection with *M. gallisepticum* is often performed by aerosol presentation of the inoculum, a method which is highly efficient and believed to be similar to the natural mode of infection (14). In an attempt to
standardise the conditions for *M. gallisepticum* challenge, a special chamber for experimental aerosol infection has been designed (143).

No published evidence is available to demonstrate the role of airborne transmission of *M. gallisepticum* under field conditions, although circumstantial evidence is often found. However, transmission of enzootic pneumonia in swine, caused by *M. hyopneumoniae*, is likely to be similar to the situation with respect to *M. gallisepticum* in commercial poultry operations. Early studies demonstrated the influence of the location of a farm on disease status, since the distance from the source of infection was identified as the major risk factor for infection of swine with *M. hyopneumoniae* (47, 133). The risk of infection rises as the proximity of susceptible animals to the source of infection increases, and as the density of the animal population in the area increases. A recent study conclusively demonstrated the presence of airborne *M. hyopneumoniae* on farms where acute respiratory disease was present (134). The organism was detected with a sensitive PCR test of air samples in the pig houses where disease problems were common.

Delineation of the lateral spread of *M. gallisepticum* from an introduced infected bird within small populations of chickens was studied by following appearance of *M. gallisepticum* serological reactions in contact birds (101). A multi-phased pattern of transmission was found, with variation in reaction among birds. Increasing the population density increased the rate of spread. Using *M. gallisepticum*-PCR or isolation to detect infection, the spread of *M. gallisepticum* from actively infected turkey poulnets to susceptible pen mates can be detected within four to seven days, although serological responses were not detected until approximately two weeks after the birds were placed in contact (S. Levisohn, unpublished findings). Live *M. gallisepticum* vaccines are transmitted more slowly among birds in the same pen, and between pens, although the biological basis for this difference is not known (65, 93).

Many *M. gallisepticum* outbreaks in breeder flocks maintained under conditions of biosecurity appear to be attributable to human factors. Some of the possible means by which this may occur in the specific cases can be identified by careful analysis of traffic patterns between flocks and behaviour patterns of the poultry farm personnel. Investigations into the survival time of *M. gallisepticum* on materials found in the poultry house environment demonstrate the resilience of this organism. *Mycoplasma gallisepticum* could be reisolated from several inert materials after two days, and survived on human hair for three days (23). Early studies demonstrated the ability of *M. gallisepticum* to survive for as long as nine days in water containing biological material (10% mycoplasma broth) (117).

Introduction of *M. gallisepticum* by free-flying wild birds that penetrate the premises of a poultry farm is often proposed as a method of transmission. *Mycoplasma gallisepticum* and *M. synoviae* have been demonstrated by specific PCR tests in wild birds, including some captured in the area of *M. gallisepticum*-infected poultry flocks, but no conclusive evidence for a role in transmission has been found (S. Levisohn, unpublished findings).

Molecular typing methods for the identification of *M. gallisepticum* strains are now being used for epidemiological studies, helping to identify the link between outbreaks occurring on different premises. Evidence has been found for infection by the same strain in different flocks within the same poultry production company (20) or among flocks in the same geographic area, including those in different poultry sectors (88, 89). Molecular typing of *M. gallisepticum* isolates has provided conclusive evidence for presence of *M. gallisepticum* vaccine strains in non-vaccinated flocks (90) (S.H. Kleven, unpublished findings).

Public health and international trade implications

No public health or zoonotic issues are associated with *M. gallisepticum*.

Since *M. gallisepticum* is transmitted by embryonated (fertile) eggs, buyers or importers of day-old breeder replacement chicks or hatching eggs should be provided assurance that the flocks from which the eggs originated are free of infection in order to prevent dissemination of the disease. Sellers should provide copies of negative serological test results from serum drawn one month or less before the eggs were produced. In the case of international exports, such test certificates should be provided by an official agency of the exporting government, such as the NPIP in the USA. For further confidence in test results, results of PCR tests taken one week or less before the hatching eggs were produced may be provided. Fresh or frozen poultry meat products or infertile eggs produced for human consumption are not ordinarily considered risks for *M. gallisepticum* infection.

Prevention and control

In most countries, control programmes for *M. gallisepticum* are based on maintenance of freedom of infection in commercial breeding stock. Voluntary *M. gallisepticum* control programmes in the USA are administered by the United States Department of Agriculture under the NPIP, testing provisions and protocols are provided in an official publication (3). *Mycoplasma gallisepticum* is included in the European Communities Council Directive (90/539/EEC) which stipulates conditions concerning trade within the
European Community and imports from third countries of poultry and hatching eggs (31).

The basic concepts of the NPIP are as follows:

a) a high level of biosecurity of breeder flocks, with production in single-aged, all-in/all-out farms
b) routine monitoring by serological testing backed up by rapid and specific confirmatory tests
c) immediate slaughter of infected breeding flocks to prevent transmission of mycoplasma to the progeny.

In general, purchasers of fertile hatching eggs or day-old poults or chicks require certification that the source breeding flock is free of M. gallisepticum infection.

However, poultry production has increased dramatically in many parts of the world, resulting in the construction of large multi-age production complexes, usually for commercial egg production, but occasionally also for breeding stock or even broilers. In addition, large populations of poultry are often reared in relatively small geographic areas, sometimes with mixed avian species or mixed types of commercial poultry maintained in close proximity. In such instances, maintenance of freedom of infection in all flocks may be difficult or impossible. Therefore, suitable antibiotic medication may be used to alleviate clinical signs and reduce production losses or egg transmission. In some situations vaccination may be an option.

Mycoplasma gallisepticum is known to be susceptible to several antibiotics, including macrolides, tetracyclines, fluoroquinolones and others (13, 82, 95), but is resistant to penicillins or other antibiotics which act by inhibiting cell wall biosynthesis. Mycoplasma gallisepticum may develop resistance against commonly used antibiotics (154). Antibiotic medication has been used in the treatment of respiratory disease (46, 55, 56, 135), to prevent egg production losses (11, 116), and to reduce egg transmission (115, 151). Although suitable antibiotic medication may reduce the severity of clinical signs and lesions, it may also significantly reduce populations of M. gallisepticum in the respiratory tract (26, 54). However, medication should not be relied upon to eliminate M. gallisepticum infection from an infected flock and should be regarded as a method for short-term amelioration of signs and economic effects, rather than as a long-term solution to the problem.

In instances where the use of infected breeder flocks for egg production is necessary, egg dipping in an antibiotic solution may be used to reduce egg transmission (50, 112). Methods of egg dipping and antibiotic injection to control M. meleagridis egg transmission in turkey eggs (42) may be suitable for elimination of M. gallisepticum egg transmission. Egg heating, an alternative method of reducing egg transmission, has also been practised (147).

In cases where control of M. gallisepticum infection is not feasible, vaccination with either inactivated or live vaccines may be an option. Vaccination as a control measure was first suggested by Adler (1) and was put into practice in the 1960s by Luginbuhl et al. (98) and by Fabricant (34). Vaccination is most commonly used in commercial egg pullets to be placed on multi-age commercial egg production sites, and in some instances, in broiler breeder pullets. The subject of M. gallisepticum vaccination has recently been reviewed by Whithear (139). With any M. gallisepticum vaccine, vaccination before exposure to wild-type infection is essential.

Inactivated bacterins have proved to be efficacious in prevention of respiratory signs and lesions in chickens (51, 124, 148, 149), and have been demonstrated to be beneficial in reducing egg production losses and egg transmission (44, 45, 51). However, other studies have shown minimal or no effectiveness. Bacterins have had a negligible effect in reducing M. gallisepticum populations in the upper respiratory tract (66) and are generally felt to be of minimal value in long-term control of infection on multi-age production sites. Since they are inactivated, bacterins are considered safer than live M. gallisepticum vaccines; however, bacterins have the disadvantages of high cost and the need to individually vaccinate each bird. Two doses are considered more effective than a single dose, but this is seldom practised because of the expense.

Three strains of live M. gallisepticum vaccine are available in different countries of the world, namely: F strain, 6/85 and ts-11, although not all countries officially permit the use of live vaccines.

The origin of the F strain is somewhat unclear, but the strain was probably isolated by Yamamoto and Adler (146) and later utilised as a vaccine (34, 98). The F strain is considered to be an M. gallisepticum strain of low to moderate virulence and transmissibility (65), but is capable of inducing respiratory signs in broilers (127) and is considered too virulent for use as a vaccine in broilers (126) or turkeys (97). The strain persists in the upper respiratory tract for the life of the flock (65). Mycoplasma gallisepticum isolates from turkeys with clinical respiratory disease have been determined to be F strain in origin (90). Vaccination with the F strain has been shown to be effective in preventing egg production losses in commercial layers (19, 27, 105). An important characteristic of F strain is the ability to induce resistance against infection by wild-type or challenge infections. In laboratory studies, the F strain significantly reduced populations of a challenge strain in the upper respiratory tract (25, 85), effectively displaced infection with a challenge strain in pen trial studies (72), and displaced wild-type M. gallisepticum in a multi-age commercial egg production site (71). The F strain vaccine can be administered by several routes including intraocular and intranasal, and by coarse spray. Vaccine is generally administered at eight to fourteen weeks of age, but can be administered as early as two
weeks or less if chicks are at risk of exposure to wild-type infection before eight weeks.

Development and characterisation of the 6/85 strain of *M. gallisepticum* live vaccine were described by Evans (32, 33). The 6/85 strain is a modified *M. gallisepticum* strain originating in the USA which is avirulent for chickens and turkeys, is not easily transmitted from bird to bird, and induces resistance against challenge with virulent *M. gallisepticum*. The vaccine has been approved for marketing in more than ten countries, in addition to the USA. The 6/85 vaccine is administered by spray, does not induce an antibody response, and can be detected in the upper respiratory tract for four to eight weeks after vaccination (32, 93). The vaccine has been used primarily for prevention of egg production losses in commercial layers in the USA.

The development and characteristics of the ts-11 *M. gallisepticum* vaccine strain have been described by Whithear et al. (140, 141, 142). The strain was developed by chemical mutagenesis of an *M. gallisepticum* isolate from Australia and was selected as a temperature-sensitive mutant. The strain is avirulent for chickens and turkeys, has a low propensity to spread from bird to bird, elicits a slow development of circulating antibody, and induces protection to *M. gallisepticum* challenge. The ts-11 strain persists for the life of the flock in the upper respiratory tract and induces long-lived immunity (139). Growing pullets are vaccinated by intraocular administration to each bird. The commercial vaccine based on the ts-11 strain is registered in Australia (the country of origin) and the USA, with registration approved or pending in approximately twenty other countries world-wide.

Both the ts-11 and 6/85 strains are characterised by lack of virulence and very poor ability to spread from bird to bird. Various types of chickens and turkeys kept in close contact across a wire barrier with vaccinated chickens for several months did not become infected with either strain, although evidence of very slight spread was found in chickens kept in direct contact with ts-11 vaccinated birds (93). Because of these superior safety characteristics of avirulence and low potential for unintended spread to nearby unvaccinated flocks, both 6/85 and ts-11 are considered to be preferable to F strain. Techniques for identification of specific *M. gallisepticum* strains from field outbreaks have demonstrated that both of these strains present significantly less risk of infecting nearby poultry flocks than F strain. Therefore, unless the wild-type is highly virulent, these strains are the preferable choices when *M. gallisepticum* vaccination is necessary.

An important characteristic of *M. gallisepticum* vaccines is the ability to induce resistance to infection from wild-type challenge, resulting in 'displacement' of wild-type strains with the vaccine strain on multi-age production sites. This characteristic could be used as a tool for *M. gallisepticum* eradication from such sites. The F strain clearly has a strong ability to displace wild-type *M. gallisepticum* strains and has been used to displace a field strain of *M. gallisepticum* on a multi-age commercial egg production site (71). Unfortunately, when vaccination on this farm was discontinued, F strain continued to cycle from flock to flock; eradication of *M. gallisepticum* was therefore not achieved. In a pen trial study, F strain demonstrated the ability to displace a virulent challenge strain when vaccinated birds were placed with chickens which had been previously challenged with R strain. However, neither ts-11 nor 6/85 exhibited an ability to displace the virulent challenge strain under the conditions of this study. In contrast, when ts-11 was used to vaccinate replacement pullets on a site previously populated with F strain, displacement of F with the ts-11 strain occurred in all vaccinated flocks. When vaccination was discontinued, *M. gallisepticum* could no longer be detected on the farm (138). Although displacement has not been extensively studied under field conditions, field data suggest that use of the 6/85 strain over several years on multi-age farms has resulted in production complexes that are serologically negative for *M. gallisepticum*, suggesting that displacement has occurred (K. Honneger, personal communication). Field experience and unpublished data suggest that when field challenge occurs with highly virulent *M. gallisepticum* strains, flocks may become infected with wild-type *M. gallisepticum* (N. Ikuta and S.H. Kleven, unpublished findings). In such cases, the use of F strain may be necessary on the site for at least one production cycle, after which the milder 6/85 or ts-11 strains could be employed.

Vaccination of turkeys has not been shown to be feasible. However, restricted use of the 6/85 strain in commercial turkeys has produced positive assessments (R.P. Chin, K. Honneger and S.H. Kleven, unpublished findings), although no reports have been published on this use of the vaccine. No reports exist of successful use of *M. gallisepticum* vaccines in broilers.

Although vaccination is considered an alternative means of control of *M. gallisepticum*, especially in commercial layers, the use of stock which is free from *M. gallisepticum* is preferable whenever possible.
Mycoplasmose aviaire (*Mycoplasma gallisepticum*)

S. Levisohn & S.H. Kleven

Résumé

*Mycoplasma gallisepticum* est un mycoplasme aviaire de distribution mondiale. C'est celui dont le pouvoir pathogène a les conséquences économiques les plus graves. À l'instar des autres mycoplasmes, *M. gallisepticum* est d'une taille minuscule, il est doté d'une information génétique minime et il est totalement dépourvu de paroi bactérienne. C'est ce qui explique l'interdépendance étroite entre *M. gallisepticum* et l'animal hôte, ainsi que les difficultés d'isoler l'organisme *in vitro*. Les souches de *M. gallisepticum* offrent des différences très nettes au regard de propriétés biologiques importantes telles que le pouvoir pathogène, l'infectiosité, le tropisme tissulaire et la transmissibilité. De plus, la variation phénotypique des principaux antigènes de surface est très fréquente ; c'est probablement la raison pour laquelle *M. gallisepticum* induit une infection chronique en dépit de l'existence d'une forte réponse immunitaire.

L'infection due à *M. gallisepticum* se manifeste par une grande variété de signes cliniques, mais même lorsqu'elle est inapparente, l'impact économique peut être important. La forme clinique la plus grave est une maladie respiratoire chronique chez les volailles destinées à la consommation ; *M. gallisepticum* est l'un des agents responsables de ce syndrome multifactoriel. La transmission *in ovo* de *M. gallisepticum* entre les sujets reproducteurs contaminés et leur progéniture est la principale voie de propagation de l'infection et un sujet de préoccupation majeur pour les échanges internationaux.

Dans la plupart des pays, les programmes de lutte contre *M. gallisepticum* consistent à préserver de l'infection les élevages de sujets reproducteurs. Dans les cas où il est impossible de mettre en œuvre une prophylaxie sanitaire, on a recours à la vaccination, notamment à l'aide de nouveaux vaccins à mycoplasmes vivants. Des progrès majeurs ont été accomplis ces dernières années en matière de diagnostic. La prophylaxie se fonde sur des programmes de dépistage sérologique utilisant l'épreuve d'agglutination sur lame ou des épreuves immuno-enzymatiques. Les réactions positives à l'épreuve d'agglutination sur lame doivent être confirmées par d'autres épreuves sérologiques et/ou par des tests démontrant la présence du mycoplasme. En principe, on peut soit isoler *M. gallisepticum*, soit identifier son acide désoxyribonucléique à l'aide de méthodes moléculaires. L'amplification en chaîne par polymérase est une méthode alternative, à la fois plus rapide et plus sensible que les méthodes traditionnelles de mise en culture qui font appel à des techniques spécialisées et longues. Les méthodes récemment développées de typage moléculaire offrent de nouvelles perspectives pour les études épidémiologiques et l'identification des réservoirs d'infection.

Mots-clés

Mycoplasmosis aviar (*Mycoplasma gallisepticum*)
S. Levisohn & S.H. Kleven

Resumen
*Mycoplasma gallisepticum*, un patógeno presente en el mundo entero, es el micoplasma que afecta con mayores repercusiones económicas a las aves de corral. Al igual que otros micoplasmas, *M. gallisepticum* es un organismo diminuto, carente por completo de pared celular bacteriana y dotado de un volumen mínimo de información genética. En la práctica, estas propiedades se traducen en un grado muy alto de interdependencia entre *M. gallisepticum* y sus huéspedes y lo convierten en un organismo muy exigente para su cultivo in vitro.

Las diversas cepas de *M. gallisepticum* presentan sensibles diferencias en cuanto a ciertas propiedades biológicas de importancia, como la patogenicidad, la infectividad, la transmisibilidad o el tropismo por ciertos tejidos. Por otra parte, los principales antígenos de superficie experimentan con gran frecuencia cambios fenotípicos, lo que seguramente explica los casos de infección crónica por *M. gallisepticum* incluso en presencia de una intensa respuesta inmunitaria. La infección por *M. gallisepticum* puede expresarse clínicamente de formas muy diversas, pero aun en ausencia de signos clínicos puede tener efectos económicos significativos. La más grave manifestación clínica de la infección por *M. gallisepticum* es la enfermedad respiratoria crónica en aves de engorde, que a menudo se presenta asociada a otros agentes etiológicos, dando lugar a un complejo patológico multifactorial. La transmisión de *M. gallisepticum in ovo* (de reproductores infectados a su progenie) constituye el mecanismo básico de propagación de la enfermedad y el principal factor que hay que tener en cuenta de cara al comercio internacional.

En la mayoría de los países, los programas de lucha contra *M. gallisepticum* están centrados en mantener libres de infección a las bandadas reproductoras de las explotaciones industriales. Para los casos en que no sea factible controlar la infección, se está evaluando la alternativa de las vacunaciones, utilizando sobre todo una nueva generación de vacunas preparadas a partir de organismos vivos. En los últimos años se han realizado notables progresos en lo que concierne a los métodos de diagnóstico. Los programas de control han venido basándose en la aplicación de técnicas serológicas en campañas de detección, sobre todo la prueba de seroaglutinación en placa y el ensayo inmunoenzimático. Para confirmar los posibles resultados positivos a la prueba de seroaglutinación es preciso aplicar nuevas pruebas serológicas y/o demostrar la presencia del microorganismo, lo que en principio es posible ya sea por aislamiento del organismo o detectando su ADN por métodos moleculares. La amplificación en cadena por la polimerasa representa una alternativa rápida y sensible a los tradicionales métodos de cultivo, que requieren la aplicación de técnicas lentas y laboriosas. La aparición de métodos moleculares de tipificación abre nuevas oportunidades para realizar estudios epidemiológicos o descubrir reservorios de la infección.

Palabras clave
References


