CEVA HANDBOOK
OF POULTRY DISEASES

6 MYCOPLASMA GALLISEPTICUM INFECTIONS

MYCOPLASMA GALLISEPTICUM INFECTIONS

DEFINITION

Mycoplasma Gallisepticum (MG) infections cause respiratory diseases in domestic poultry and other birds. Their primary clinical and morphological manifestations are chronic respiratory disease in chickens, infectious sinusitis in turkeys and conjunctivitis in finches.

HISTORY

The first reports of infection in turkeys came from England. In 1905 Dodd observed oedemas in the region of the head in turkeys housed together with other birds, which did not exhibit these symptoms. He managed to isolate a cholera-like organism from the swellings and the internal organs and described the condition as "epizootic pneumoenteritis of the turkey". Two years later further instances of" swollen head" in turkeys were reported by Graham-Smith, again in England. He managed to make a transmission with manifestation of sinusitis via inoculation of exudate from the sinuses, and to isolate a non-specific organism (Lancaster & Fabricant, 1988). In the USA, sinusitis in turkeys was described for the first time by Tyzzer in 1926 (Yoder, 1963). Infectious sinusitis in turkeys was studied in detail in 2 flocks in 1946, and it was assumed that the etiological agent was a virus (Jerstad & Hamilton, 1948). There is very little doubt that the coccobacilliform bodies observed and described for the first time by Nelson were, in fact, *Mycoplasma gallisepticum* (Lancaster & Fabricant, 1988). He associated these bodies with coryza, which has different characteristics, depending on its development.

The term "chronic respiratory disease" was first mentioned in the title of a scientific report (Delaplane & Stuart, 1943). The publication presented an illness in chickens with respiratory signs and a slow spread rate. The microbiological work conducted in relation to the case led the authors to believe that the causative agent was different from the viruses behind infectious bronchitis or laryngotracheitis. In 1954, a respiratory disease in hens affecting the air sacs, which was associated with coccobacilliform bodies similar to the ones observed by Nelson, was described in England (Chu, 1954).

In the early 1950s it was established that the infectious sinusitis in turkeys and chronic respiratory disease in chickens was a pleuropneumonialike organism (PPLO), (*Mycoplasma spp.*),

(Markham & Wong, 1952; van Roekel & Olesiuk, 1953). Another important step towads diagnosis was the differentiation of pathogenic and non-pathogenic PPLO (Adler & Yamamoto, 1957). The term PPLO was predominantly used until the 1960s, after which the name *Mycoplasma gallisepticum* was proposed to designate the organism that causes infectious sinusitis in turkeys and chronic respiratory disease in chickens (Edward & Kanarek, 1960).

Further important episodes in the history of *Mycoplasma gallisepticum* infections were the establishment of the role of secondary pathogens, such as *E. coli* and some viruses, which can lead to acute aerosacculitis, or the so-called "complicated chronic respiratory disease," the development of techniques for isolation and serological methods for diagnostics of *Mycoplasma* infection, and detection of the important role of eggs in the transmission of *Mycoplasma* infections (Lancaster & Fabricant, 1988).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

Mycoplasma gallisepticum belongs to the family Mycoplasmataceae, order Mycoplasmatales, class Mollicutes, genus Mycoplasma. Mycoplasmas (Mollicutes) are phenotypically different from other bacteria, with their tiny size and lack of a cell wall. They are characterised by an obligate parasitic way of life, highly dependent on the host animal (Levisohn & Kleven, 2000). The Mycoplasma's fastidious nature determines its requirements in terms of nutrient media during in vitro culturing. The lack of a cell wall makes the organisms highly pleomorphic and hard to detect using light microscopy of Gram-stained imprint preparations. The specific "fried egg" morphology results from the appearance of tiny colonies among the fibres of the nutrient medium (Levisohn & Kleven, 2000). The lack of cell wall in Mycoplasmas, however, allows for the use of antibiotics acting upon the cell wall, in order to suppress other organisms in the culture medium (Kleven, 1998).

Mycoplasma gallisepticum was differentiated from the other *Mycoplasma* affecting birds through serotyping and was classified as serotype A (Kleckner, 1960; Yoder, 1964). Mycoplasmas with similar antigenic or phenotype properties to MG have been differentiated using molecular techniques and designated as *M. imitans* (Kleven, 2008). There is an apparent variability between and within the strains (Bencina, et al., 1994; Garcia et al., 1994). Based on the variable antigens and biological differences, some strains have been singled out as variants or "atypical." Comparisons can thus be made between the live vaccinal strains F, ts-11 and 6/85 (Levisohn & Kleven, 2000). Electron microscopy has shown that *M. gallisepticum* organisms are round with an approximate size of 0.25 – 0.5 µm. They may exhibit filamentous structures, which are related to motility, haemotaxis, and pathogenicity (Balish & Krause, 2005). The culture media for *M. gallisepticum* are more specific and require the addition of horse or other serum and penicillin. Incubation takes 3–5 days or more in aerobic conditions. Biochemically, the Mycoplasmas cause complete haemolysis of erythrocytes in horse blood agar and agglutinate turkey and chicken erythrocytes.

MG organisms are inactivated by commonly used chemical disinfectants such as formalin, phenol, etc. The organism's resistance at 5–10°C in poultry barns is up to 4 weeks. In bird faeces at 20°C, they remain vital for 1–3 days, in egg yolk for up to 6 weeks at 20°C, and for up to 18 weeks at 3°C. They can survive for 2–4 days in bird feathers and up to 3 days in human hair (Kleven, 2008). In laboratory conditions, they can survive for 30–40 days stored at 2–4°C, and in lyophilised state: for 7–14 years (Obreshkov et al., 1978).

EPIDEMIOLOGY

Mycoplasmas tend to be highly host-specific and their differentiation requires specific diagnostic methods. Avian Mycoplasmas do not infect other mammals, including humans, even though there have been occasional reports of isolates from domestic animals.

MG can infect various bird species, yet gallinaceous birds are most susceptible. Under commercial conditions, hens and turkeys are affected the most, although MG has been isolated from naturally infected pheasants, guinea fowl, quails and partridges. Pigeons are not very susceptible and waterfowl are not at all affected by MG infection.

M. gallisepticum is spread throughout the entire world and is a major problem for the poultry breeding industry. Infected birds remain carriers of the organism for the rest of their lives and can remain asymptomatic until stress occurs, such as a change of premises, diet or weather, vaccinations against or infections with IB or ND, increased levels of dust or ammonia etc. Birds of all ages are susceptible, yet the young are more vulnerable. In broiler chicken production, MG infection is most commonly observed between 3 and 6 weeks of age. In layers and breeders it occurs in the period around the beginning of egg laying.

Both clinically ill birds and asymptomatic carriers are sources of the infection. Potential reservoirs of the infection include farms with mixed-age flocks, backyard birds and some wild songbirds (Fisher et al., 1997; Ferguson et al., 2003; Ley et al., 2006).

Infection transmission can happen vertically or horizontally. Vertical, or transovarial transmission, occurs through the eggs of naturally infected turkeys and hens. Transmission is most intensive during the acute stage of the disease, when the levels of MG in the respiratory tract are at their highest (Glisson & Kleven, 1984).

Horizontal transmission occurs easily among susceptible birds and clinical or subclinical carriers, through direct or indirect contact. Other factors contributing to horizontal transmission are contaminated dust, feathers, personnel, equipment, etc. High flock density at the farm, and large flock size, also facilitates horizontal spread.

Predisposing causes provoking the occurrence of the illness are mostly related to the microclimate within the buildings: inadequate ventilation, increased humidity or dust pollution, sharp temperature variations, etc.

Predilection sites for the Mycoplasmas are the mucous coats of the respiratory tract, the urogenital tract and the joints. Adhesion to host cells is a necessary condition for successful colonisation and subsequent

pathogenicity. *M. gallisepticum* is one of the Mycoplasmas responsible for acute and chronic illness with a broad range of complications (Levisohn & Kleven, 2000). By itself, the MG infection occurs in a mild, even subclinical form. Complicated *Mycoplasma* infections, however, are often encountered in commercial flocks and are a real challenge in terms of both diagnostics and treatment (Bradbury, 1984).

CLINICAL SIGNS AND PATHOLOGY

The clinical and morphological manifestation of *M. gallisepticum* infection in industrial poultry production consists primarily of infectious sinusitis in turkeys and chronic respiratory disease in chickens. In natural conditions it is very difficult to track the time from the moment of infection to the onset of the first symptoms. In experimental infections, symptoms of sinusitis in turkeys were established within 6–10 days, yet this period could vary up to 21 days (Kleven, 2008).

Infectious sinusitis in turkeys

It is characterised by a serous catarrhal to fibrinous rhinitis, conjunctivitis and sinusitis. The birds become depressed and their feed consumption and daily growth rate decrease, with ensuing weight loss. Clinical signs, morbidity and mortality rates can vary significantly. Up to 80–90% of the entire flock can be affected within a week.

Fig.1

Infectious sinusitis in turkeys. Unilateral or bilateral swelling of periorbital sinuses, nasal discharge and conjunctivitis are observed.

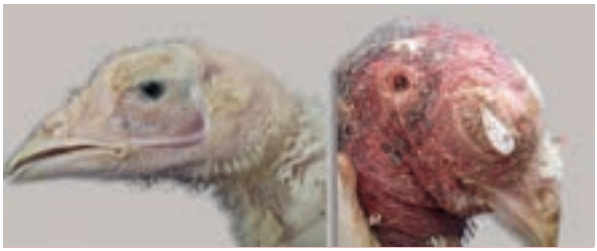

Fig.2

The inflammatory exudate is usually serous in the beginning, sometimes mixed with air bubbles and can be found in the sinuses after removing the skin.

Fig.3

In advanced cases, the exudate becomes serofibrinous.

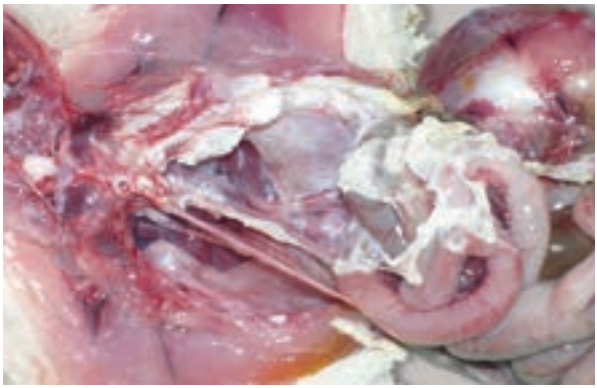

Fig.4
More rarely, air sacs and other serosas in turkeys are affected.

Chronic respiratory disease chickens
It is characterised by serofibrinous inflammation of the air sacs, the lungs, the mucous membranes of the upper respiratory tract and the conjunctiva. The occurrence of CRD in chickens results from the apparent tendency of MG to interact with other pathogens, primarily E. coli. The synergetic effect of the interaction between MG and other pathogens, such as viruses or vaccinal strains of Newcastle disease and infectious bronchitis, as well as M. synoviae, has also been demonstrated (Kleven, 1998). In broilers, the mortality rate can vary from low in non-complicated cases to 30% in cases of complications caused by the afore mentioned pathogens (Kleven, 2008). There are also collateral losses from stunted growth and pre-slaughter culling. Mortality can be low in layers, yet there is a drop in egg-laying capacity.

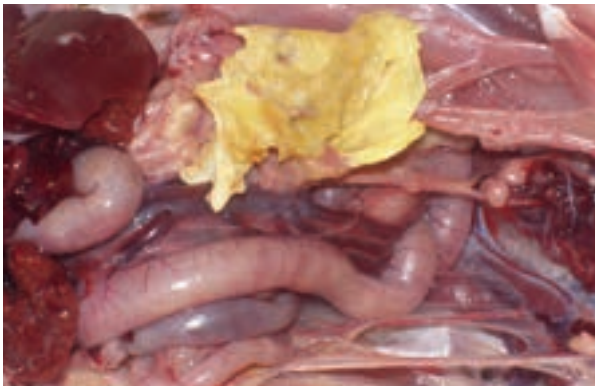

Fig.5
The most common major finding is aerosacculitis.

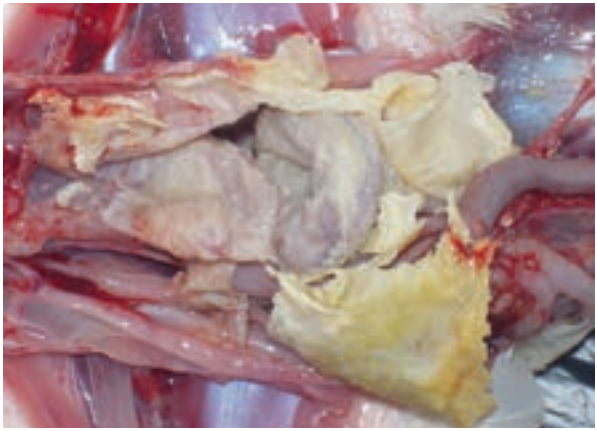

Fig.6
The air sacs are filled with fibrinouscaseous exudate.

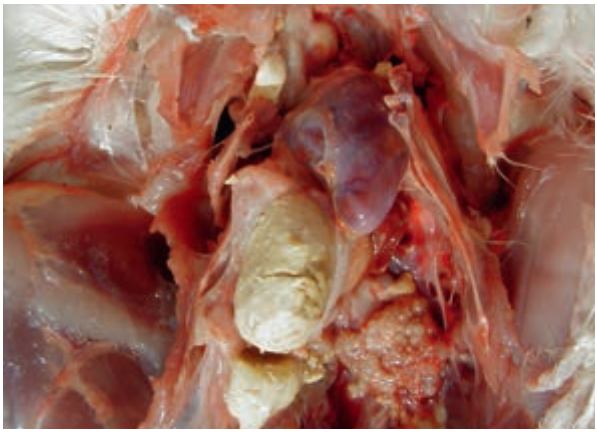

Fig.7
In older cases, the content of air sacs is dense and compact. Loss of production may occur in laying hens.

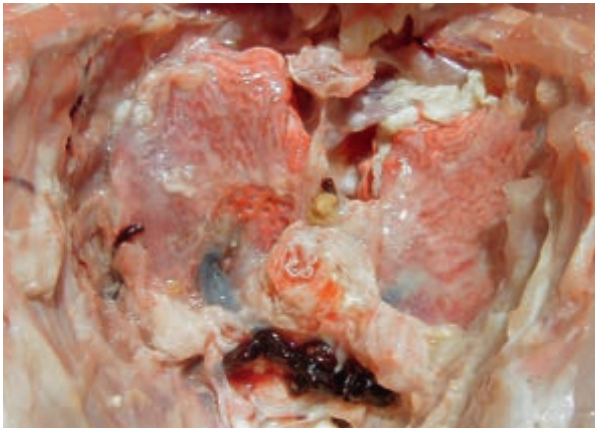

Fig.8
Serofibrinous pneumonias, usually bilateral, are a frequent finding.

Fig.9
The inflammation often affects the adjacent serous coats, and thus fibrinous polyserosites occur.

Fig.10
Sinusites are relatively rarely observed in hens.

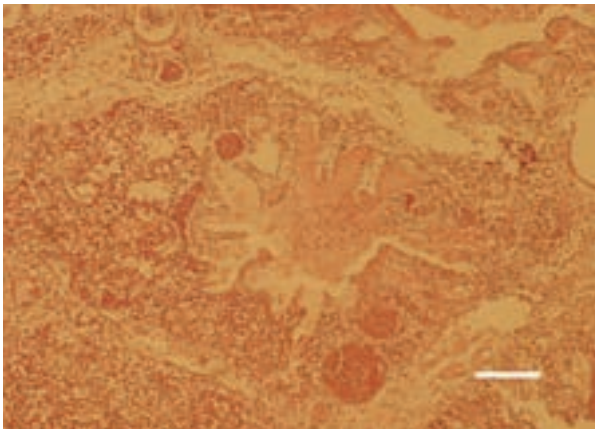

Fig.11
CRD, chicken. Congestion and diffuse heterophilic infiltration in the lung parenchyma. The lumen of parabronchi is filled with serous exudate. H/E, Bar = 50 µm.

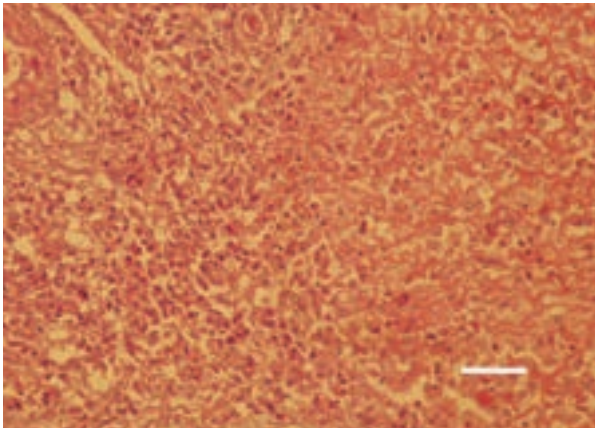

Fig.12
CRD, chicken. Airsacculitis, a transverse cross-section of the air sac, broiler chicken. Mixed inflammatory cell exudate and fibrinouscaseous masses. H/E, Bar = 35 µm.

DIAGNOSIS

The history, clinical and epidemiological characteristics of the condition and results of gross examination allow for a tentative diagnosis. Confirmation of the presence of *M. gallisepticum* can be achieved by isolating the organism in a cell-free medium or through direct detection of its DNA in swab samples or infected tissues. Suitable samples for examination can be inflammatory exudate from the air sacs, lungs, trachea, nasal sinuses, or tissue samples. In live birds, as well as from fresh or refrigerated carcasses, exudate swabs from the aforementioned areas may be collected. The samples must be obtained before the flock is subjected to antimicrobial therapy. If the time necessary for the samples to reach the laboratory is under 24 hours, it is advisable to use a cooler to maintain a temperature of 4°C. Frozen material can be used as well (tissue samples or exudate), but it should be transported in dry ice before its arrival at the lab (Kleven, 2008).

The methods of cultivation and identification of *M. gallisepticum* have been described in detail and can be found in the OIE Terrestrial Manual, chapter 2. 3. 5. 2008. The identification and differentiation of *M. gallisepticum* from other *Mycoplasma* isolates is usually based on immunological assays, most often immunofluorescence tests (Levisohn & Kleven, 2000). To identify field isolates, direct or indirect immunofluorescence is primarily used. The immunoperoxidase test can be used for rapid identification, alone or in combination with the immunofluorescence test (Bencina & Bradbury, 1992). For this purpose, growth inhibition test and nucleic acid detection methods can also be used (Clyde, 1983; Dohms et al., 1993).

A widely used diagnostic method is detecting MG-specific DNA through PCR, using commercial kits. This diagnostic method is much faster and more precise, compared to the methods of isolation and identification of the agent (Kleven, 2008).

Serological examinations are the basis of the programmes for flock monitoring and control. They include serological screening of the breeder flocks, which starts before egg-laying and continues at predetermined time periods. The traditionally used methods are commercially available ELISA kits, the haemagglutination inhibition test and the tube agglutination test (Levisohn & Kleven, 2000).

DIFFERENTIAL DIAGNOSIS

MG infections should be clinically and morphologically distinguished from other respiratory infections of viral or bacterial etiology.

Infectious sinusitis in turkeys must be differentiated from turkey rhinotracheitis (TRT), chlamydiosis, avian influenza, pasteurellosis, Ornitobacterium rhinotracheale (ORT) infection etc. Chronic respiratory disease in chickens should be differentiated from swollen head syndrome in chickens (SHS), ORT, fowl cholera etc.

Regarding conjunctivitis in finches, chlamydiosis and other bacterial infections should be taken into consideration.

PREVENTION AND CONTROL

Programmes for prevention and control of MG infections are primarily aimed at maintaining pathogen-free commercial flocks. This is currently achieved through strict biosecurity measures and serological monitoring of flocks, as well as the methods for isolation and identification of the etiological agent described above. These measures are successful to a great extent in reducing the spread of MG among breeder flocks of turkeys and hens. Moreover, compliance with the fundamental "all in – all out" principle, applied to meat-type flocks of turkeys and broilers, can lead to complete eradication in affected flocks. At the same time, this method has proved unsuccessful in farms with mixed-age layer flocks in which, due to the high density of birds, adhering to this principle is impossible (Evans et al., 2005). The EU Directive on combating MG infections in birds includes several basic concepts such as: high level of biosecurity in the breeder flocks with production in single-age "all in – all out" farms; routine serological monitoring; immediate eradication of infected breeder flocks to prevent the spread of the pathogen to the offspring. An important requirement for retail breeder eggs is to declare via an appropriate certificate that the flock of origin is free of MG (Levisohn & Kleven, 2000).

Common problems which make the eradication of the MG infection difficult or impossible, however, include highly-concentrated poultry production conditions, especially in specific regions, farms that are home to various species or different bird types, as well as the close proximity of backyard birds, which can serve to maintain the infection. In such cases, an appropriate antibiotic therapy can be applied in order to reduce losses from morbidity, mortality or ovarian transmission. It should be however remembered that antibiotics suppress but do not eliminate MG infections. Moreover increasing resistance to the commonly used anti-infectives has been described.

Vaccines

Vaccines can be used for the prevention of respiratory infection in commercial layers, as well as for the eradication and reduction of transmission through eggs in breeder flocks.

The first commercially available vaccines were oil-emulsion bacterins. Bacterins do not pose a risk to biosecurity (dead organisms),

yet they are not very efficient for farms with endemic MG infections. Moreover, they reduce but do not eliminate MG colonisation in subsequent infections. Another difficulty is the necessity for administration of 2 doses for optimal protection, via individual application. Live MG vaccines include the vaccinal strains F, 6/85 and TS11. The F strain (Connecticut) is a relatively mild strain, which reduces but does not eliminate the possibility for transmission through eggs. It effectively replaces the infection with field strains. Flocks vaccinated with MG F maintain the organism in the upper respiratory tract throughout the flocks entire life. It is pathogenic to turkeys. The vaccine is recommended for application at the age of 8–14 weeks in the form of eye or nasal drops or coarse spray. MG 6/85 is minimally virulent in chickens and turkeys, and there is little or no bird-to-bird transmission. Aerosol application is recommended after 6 weeks of age. It can be detected in the upper respiratory tract 4–8 weeks after vaccination. MG TS 11 (Australian field isolate). Minimum or no virulence in turkeys and chickens, weak transmission from bird to bird. In vaccinated flocks, TS 11 persists throughout the entire life in the upper respiratory tract and induces long-term immunity. The recommended route of application is a single dose via eye drops in pullets after 9 weeks of age.

An important characteristic of live vaccines is their ability to induce protection and to compete with (and to replace) wild MG strains in farms and mixed-age flocks. Following the cessation of vaccination, however, MG F continues to circulate from flock to flock and eradication is impossible (Kleven et al., 1990; Levisohn & Kleven, 2000). There are numerous reports of isolating MG 6/85 from turkeys with clinical symptoms which were located in proximity to vaccinated hens (49-th Annual New England Poultry Health Conference, 2000).

TS 11-like isolates have been found in 2 cases of non-vaccinated hen flocks (49-th Annual New England Poultry Health Conference, 2000). The application of a live MG vaccine is allowed by the legislation where it is approved, adhering strictly to the producer's recommendations and taking into consideration the safety of unaffected flocks (Kleven, 2008).

The development of new MG vaccines nowadays is based on the technological advantages of

molecular biology. Progress in this field makes the application of MG and other *Mycoplasma* species of the same genus possible in the development of recombinant vaccines . With this new technology, safe and efficient MG control for all birds is expected to become a reality in the near future (Evans et al., 2005).

TREATMENT

Antibiotics, which affect the cell wall, have no effect on Mycoplasmas. Penicillins and cephalosporins should not be used. Mycoplasmas are sensitive to tetracyclines, quinolones, tylosin, tiamulin, tilmicosin.

REFERENCES

- Adler, H.E. & R. Yamamoto, 1957. Pathogenic and nonpathogenic pleuropneumonia-like organisms in infectious sinusitis of turkeys. Am J Vet Res., 18, 655-656.
- Balish, M.F. & D.C. Krause, 2005. Mycoplasma Attachment Organelle and Cell Division. In A. Blanchard and G. Browning Gliding Motility of Mycoplasmas: The Mechanism Cannot be Explained by Current Biology, Wymonbham, UK: Horizon Bioscience, 189-237.
- Bencina, D. & J.M. Bradbury, 1992. Combination of immunofluorescence and immunoperoxidase techniques for serotyping mixtures of Mycoplasma species.Jour of Clin Micro., 30, 407-410.
- Bencina D., S.H. Kleven, M.G. Elfaki, A. Snoj, P. Dovc, D. Dorrer & I.Russ, 1994. Variable expression of epitopes on the surface of Mycoplasma gallisepticum demonstrated with monoclonal antibodies. Avian Pathol., 23, 1, 19-36.
- Bradbury, J.M., 1984. Avian mycoplasma infections: prototype of mixed infections with mycoplasmas, bacteria and viruses. Ann Microbiol. /Inst. Pasteur/, 135A, 83-89.
- Chu, H.P., 1954. The identification of infectious coryza associated with Nelson`s cocco-bacilliform bodies in fowis in England and its similarity to the chronic respiratory disease of chickens. Proc. 10th World`s Poult. Congr., 2, 246-251.
- Clyde, W.A., 1983. Growth inhibition tests. In S. Razin and J.G. Tully Methods in Mycoplasmology Vol. 1. Mycoplasma Characterization. New York, N.Y.: Academic Press, 405-410.
- Delaplane, J.P. & H.O. Stuart, 1943. The propagation of a virus in embryonated chicken eggs causing a chronic respiratory disease of chickens. Am J Vet Res., 4, 325-332.
- Dohms, J.E., L.L. Hnaiow, P. Whetzei, R. Morgan & C.L. Keeler, 1993. Identification of the putative cytadhesin gene of Mycoplasma gallisepticum and its use as a DNA probe. Avian Dis., 37, 380-388.
- Edward, D.G. ff. & A.D. Kanarek, 1960. Organisms of the pleuropneumonia group of avian origin: their classification info species. Ann N Y Acad Sci., 79, 696-702.
- Evans, J.D., S.A. Leigh, S.L. Branton, S.D. Collier, G.T. Pharr & S.M.D. Bearson, 2005. Mycoplasma gallisepticum: Current and Developing Means to Control the Avian Pathogen. Poultry Sci Ass Inc., 757-763.
- Ferguson, N.M., D. Hermes, V.A. Leiting & S.H. Kleven, 2003. Characterization of a naturally occurring infection of a Mycoplasma gallisepticum house finch-like strain in turkey breeders. Avian Dis., 47, 523-530.
- Fischer, J.R., D.E. Stallknecht, P. Luttrell, A.A. Dhondt & K.A. Converse, 1997. Mycoplasmal conjunctivitis in wild songbirds: the spread of a new contagious disease in a mobile host population. Emerg Infect Dis., 3, 69-72.
- Garcia M., M.G. Elfaki & S.H. Kleven, 1994. Analysis of the variability of Mycoplasma gallisepticum surface antigens. Vet Mic., 42, 2-3, 147-158.
- Glisson, J.R. & S.H. Kleven, 1984. Mycoplasma gallisepticum vaccination: effects an egg transmission and egg production. Avian Dis., 28, 406-415.
- Jerstad A.C. & C.M. Hamilton, 1948. The etiology of infectious sinusitis of turkeys. Poult Sci., 27, 802-812.
- Kleven, S.H., 1998. Mycoplasmosis. In: A laboratory manual for the isolation and identification of avian pathogens, 4th Ed., American Association of Avian Pathologists, Kennett Square, Pennsylvania, 74-80.
- Ley, D.H., D.S. Sheaffer & A.A. Dhondt, 2006. Further western spread of Mycoplasma gallisepticum infection of house finches. J Wildl Dis., 42, 429-431.

- Levisohn S. & S.H. Kleven, 2000. Avian mycoplasmosis / Mycoplasma gallisepticum/. Rev Sci Tech Off Int Epiz., 19, 2, 425-442.
- Markham, F.S. & S.C. Wong, 1952. Pleuropneumonia-like organisms in the etiology of turkey sinusitis and chronic respiratory disease of chickens. Poult Sci., 31, 902-904.
- Obreshkov, K., I. Vasilev, B. Natchev, et al., 1978. Diseases of poultry, Zemizdat, Sofia, pp. 119-128.
- Van Roekel, H. & O.M. Olesiuk, 1953. The etiology of chronic respiratory disease. Proc. 90th Ann. Meet. Am. Vet. Med. Assoc. pp. 289-302.
- Yoder, H.W., 1963. Characteryzation of avian mycoplasma. Ph.D. Thesis, Iowa State Univ. Avian mycoplasmosis (Mycoplasma gallisepticum), OIEI, 2007. http://www.cfsph.iastate.edu/Factsheets/pdfs/avian_mycoplasmosis_mycoplasma_gallisepticum.pdf