Past, present and future of *M. gallisepticum* infection

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The past of *M. gallisepticum* infection

The most economically important mycoplasma species in poultry is *M. gallisepticum.* There are a great variability between strains in respect of tissue tropism (proclivity for brain, eye, join), culturing ability, virulence and transmissibility and antigen structure. This mycoplasma can cause diversity of clinical signs: chronic respiratory disease, downgrading of carcasses, decrease of egg production, hatchability, growth rate, increase of mortality and feed conversion efficiency. It induces gross and histological pathological legions in air sacs, peritoneum and oviduct, more rarely in eyes or brain. *M. gallisepticum* is frequently present as one of major agents in multi factorial disease complex. *M. gallisepticum* infection predispose birds to action of *Escherichia coli, Haemophilus paragallinarum*, vaccine strains of Newcastle diseases or infectious bronchitis and laryngotracheitis viruses, to various stress factors of environment (as increased or decreased temperature, higher concentration of ammonia and dust in air). It should be stressed that the above-mentioned factors might have economical impact in presence or in absence of clinical of signs.

M. gallisepticum infection can spread vertically from hens to progeny and horizontally by direct contact of susceptible birds with infected carriers (or by airborne route) and by indirect contact by contaminated environment. Infection may occur basically in chickens and turkeys, but it can be detected in variety of game birds (partridges, pheasants, quails, red-legged partridges), wild birds, (finch, goldfinches, blue jay, song birds, pigeons) being closely to infected flock. Therefore these birds can serve always as infection source for chickens and turkeys.

In the horizontal transmission of the infection multi-phased pattern of transmission was detected. Transmission occurs within 4-7 days. Increasing the population density increased the rate of spread. Human factors are very important in spread of infection since the survival time of *M. gallisepticum* on materials in poultry house and environment is about 2 days, on human hair is 3 days but in egg yolk and egg white is about 10-12 days.

The main route of infection of birds by mycoplasma is via the air. Mycoplasmas enter the respiratory tract and can attach to cilia and surface of the epithelial cells. Due to production of various metabolites, toxic substances and depletion of amino acids, fatty acids and DNA precursors by mycoplasmas, the normal function of the epithelial cells of the mucosal membrane of the respiratory tract is disturbed. Excretion and antimicrobials effect of mucus is changed, motility of cilia is decreased or stopped even destruction of cilia can be observed. Due to these factors mycoplasma can move down to the lung and air sacs causing their damage. Bacteria being present in upper respiratory tract (e.g. *H. paragallinarum* or *E. coli* (E. c.)

can follow mycoplasmas, aggravating pathological processes initiated by mycoplasmas.

From the respiratory tract mycoplasma can penetrate into the blood. During the mycoplasmaemia phase, mycoplasmas can spread all over the bird including joints, ovary and oviduct and other organs. Due to this, lameness can be observed. The function of the ovary is decreased; many of the follicles, eggs can be contaminated by mycoplasmas. As a result, egg production is decreased, embryo mortality is increased.

One important feature of *M. gallisepticum* infection is that mycoplasma can persist in bird during all live, even in the presence of the humoral antibodies.

Present of *M. gallisepticum* infection

Mycoplasmas are microorganisms distinguished phenotypically from other prokaryotes by their characteristic properties, as minute size, total lack of cell wall, minimal amount of genetic information and so on. Mycoplasmas are in very close association with host cells on the surface producing several molecular biological, later on histological changes of them, opening the door for other secondary bacterial infections. Pathogenic mycoplasmas are capable of active cell invasion causing systemic infection. During mycoplasma infections the defence mechanism of the immune system is very much depressed. Normally macrophages catch penetrated antigens, depredated it by their enzymes. In case of mycoplasma infection, since

mycoplasmas adsorb a lot of host protein on their surface they are not recognised as foreign body, mycoplasmas can attach to macrophages; they can destroy complement- and antibody-fixing receptors and various other receptors on surface of macrophage. Therefore macrophages cannot destroy mycoplasmas; they cannot digest bacteria penetrating after mycoplasmas. Mycoplasmas penetrate into the peripheral immune organs, damaging surface receptors of B- and T-lymphoblasts too. This leads to delayed differentiation of B- and T-lymphocytes, to weak immune response to mycoplasmas and to increases sensitivity of infected birds to various bacterial infections. Therefore in case of mycoplasma infection incidence of penetration of bacteria (e. g. *E. coli*) into inner organs of poultry (colisepticaemia) is much high

These characteristics predispose to several practical problems in diagnostic and control of mycoplasma infections.

1.The fastidious nature of mycoplasmas causes difficulties in culturing and identification of mycoplasmas.

2. Antigen variation and modification creates difficulties in serological diagnosis. The HI test based on HI antigen is very much strain specific. Similar problem are recognised in SPA or ELISA or antigen preparation for standard serological tests. Detectable serological response is generally week. According to our experience the monoclonal based blocking ELISA has much more advantage then indirect ELISA: 1./ it is not necessary to dilute sera to be tested, therefore you can detect more antibodies then by indirect ELISA using diluted sera, 2./ the test can detect also infection due to variant

strains (with low immunogenicity), 3./ it can be used for detecting antibodies in several animal species.
Using Western blot technique provides important information about time of infection. Appearance of antibodies against P67 polypeptide only indicates of infection of one week before. Appearance of antibodies against P56 shows infection time about 2 weeks..

3. Due to close association with host cell mycoplasmas have an opportunity for resisting host immune defences. It is the reason why it is not possible to eradicate mycoplasmas infection by antibiotic treatment form chickens.
Mycoplasmas can exist in the host when antibodies are produced.

To prevent economical losses in poultry flocks control programmes against mycoplasma infection should be applied. One of the control methods is using vaccines. Vaccination induces a protectivity if it is done before infection. However, the killed vaccines, like M.g. bacterins, induce rather short (few month) immunity. Living vaccines (F, ts-11, 6/85 strains) give economical benefit and may replace wild stain but they might pose a hazard to other flocks, mainly for turkey flocks, or may aggravate the pathological processes in association with regularly used living virus vaccines. The use of vaccines also disturbs serological survey of natural mycoplasma infection.

4. Due to lack of cell wall, antibiotics, inhibiting cell wall synthesis, cannot be used for therapy of birds. Several drugs inhibiting protein synthesis, like macrolides (erythromycin, tylosin tartrate, spriramycin, kitasamycin, lincomycin), tetracyclines (oxytetracycline, chlortetracycline, doxycycline) and pleuromutilin (tiamulin hydrogen fumarate) were proposed. Drugs

effecting DNA synthesis (norfloxacin, enrofloxacin, danofloxacin) were also introduced.

Taking into consideration of the pathogenesis of mycoplasma infections another important aspect is the pharmacokinetic properties of the drug selected. It is very important the level and persistence of the compound in the target tissue, such as in the respiratory tract. Due to proper treatment of infected flocks clinical improvement, reduction of mortality, the incidence and severity of pathological lesions and mycoplasma infection rate, and increased performance (body weight, egg production) can be observed. Studies showed that combination of drugs (e. g. Tiamulin with chlortetracycline) increases efficacy of medication due to enhancement or synergistic effect of drugs against mycoplasmas and bacteria. At the same time the antibiotic spectrum of drug combination became broader in comparison with efficacy of single drugs.

5. In case of mycoplasma infection immune system is damaged significantly. This leads to delayed and weak immune response to mycoplasmas and sensitivity of infected birds to various viral and bacterial infections. Efficacy of various vaccine s is much less pronounced in mycoplasma infected flocks than in non-infected once. Due to suppression of immune system secondary viral or bacterial infections (*E.coli*) are very important, again which it difficult to immunize the birds. We have demonstrated a direct correlation between *M.gallispeticum* and *E. coli* infections: 1. In presence of *M. gallisepticum* infection occurrence of airsacculitis due to *E. coli* can reach about 25-30 %, while in flock without *M. gallisepticum* infection, it is only about 5 %. 2. In *M. gallisepticum* infected flocks *E. coli* spread much faster

than in negative flock. 3. There is a correlation between number of *E. coli* in air sac and severity of the air sac lesions. 4. Therefore in case of mycoplasma infection incidence of penetration of bacteria (e. g. *E. coli*) into inner organs of poultry (colisepticaemia) is much higher and the lesions are much more severe, higher mortality of birds can be observed than in M. *gallisepticum* free chickens. 5. In *M. gallisepticum* infected hens drop of egg production and increase of *E. coli* egg transmission is significant. *E. coli* infection alone can cause a temporary decrease of egg production with a relatively short egg transmission of *E. coli*.

According to our studies treatment of mycoplasma infected chickens with immunomodulator (e. g. Inmunair) in combination with antimycoplasma drugs significantly increases efficacy of control of mycoplasma infection.

There are several new Informations about Immunology of Mycoplasma Infection:

a. The Interaction of pathogen with the host is based on recognition of pathogen association molecular patterns (PAMs) by pathogen recognition receptors (PRRs). Among latest the Toll -like receptors (TLR) are the most important. There are 10 TLRs currently Identified. Each recognizes one or more specific ligands. For Instances, TLR-1 reacts with lipopeptides from Gram+ bacteria, TLR-2 binds bacterial lipoproteins/ lipopeptides from Gram+ bacteria as well as mycoplasmas. It is able to form heterodimers with TLR-1 and TLR-6. TLR-3 recognizes double stranded viral RNA, TLR-4 recognizes lipopolysaccharides from Gram- bacteria and lopoteichoic acid from Gram+ bacteria. TLR-5 is a receptor for flagellin from Gram- and +

bacteria. TLR-6 binds different lipoproteins of bacteria. TLR-7 and TLR-8 respond to single stranded RNA. TLR-9 recognizes unmathylated bacterial DNA (CpG DNA oligonucleotide). Each TLR has an extracellular leucine-rich domain and an Intracellular portion (Toll/IL-IR=TIR domain). In response to pathogen binding TIR domain recruits adaptor molecules on the cytoplasmic side of activated TLR and initiates the activation of NF-kB factors and expression of various cell surface proteins and mediators of inflammation. In chickens two types of TLR were cloned which have 46.3% and 80.7% homology with human TLR-2.

b. It has been recognized that the dendritic cells (DC) having several TLRs, are essential to initiated immune response. Upon activation of TLRs, inmature DCs differentiate into mature antigen representing cells which are able to present antigen in MHC class-II and class-I molecules and regulate the expression of surface co-stimulatory molecules. Mature and activated DC express CD80/CD86 molecules, migrates to the secondary lymphoid organs. CD80/CD86 molecules interact with CD28 of CD4+ T cells depending on cytokines and other molecules. This activation initiate differentiation of antigenically naive T cells to antigenically determined Th1 or Th2 cells. Th1 type response is mediated by Il-12, IL-23 and IL-24 cytokines and characterized by production of IFN-gamma and generation of IgG2 isotope antibodies. Th2 type response are driven by anti-inflammatory cytokines, such as IL-4 and IL-10 and production of antibodies IgG1 subclass antibodies.

c. Lipid associated membrane proteins from mycoplasmas interact with TLR-1, TLR-2 and TLR-6. Mycoplasmal lipopeptides induce release of

TNF-α, responsible for inflammation and IL-10. Furthermore, it stimulates proliferation and infiltration in tissues of authologus lymphocytes (This is an explanation of histological and macroscopical lesions). This results in inpair of immunity against mycoplasma infection and other associating agents.

d. DC have a special receptor, called DC-SIGN (ICAM-grabbing non-Integrin). Recently it was demonstrated that DC-SIGN preferentially recognizes high mannose olygosaccharides being present in mycoplasmas. This results In Induction of IL-10 which inhibits IL-12 production and generation of Th1 response. This make possible for mycoplasmas to escape the host defense mechanism and establish chronic Infection.

e. We have demonstrated that by removing of mannose oligosaccharides from mycoplasma antigen, we have increased immunity not only preventionally inducing it before challenge but also therapeutically, administrating it post infection. In the first case efficacy of protection based on the decrease of lesions scores and reisolation of mycoplasma from inner organs proved to be 58.6% and 67.7 %, while in the second case the efficacy was 89.3% and 91.7 %.

Future of *M. gallisepticum* infection:

1. Genetic Improvement of breading stock is based on selection and breeding of different population of birds. Therefore excluding presence of mycoplasma infection in such population is crucial. Antigenic and genetic changing of mycoplasmas makes it difficult the molecular biological, cultural and serological diagnosis of mycoplasma infections. Economical

situation hinders to perform epidemiological prevention measurements. Therefore it is expected reinfection of breeding stocks time to time.

2. General tendency to reduce antibiotic treatment of poultry flocks, permanent increase of antibiotic resistance of mycoplasmas strains being present in infected flocks as well as slow development of new anti mycoplasma drugs make it difficult to perform mycoplasma eradication programs and control of mycoplamsa infections.

3. Based on research achievements on function of DC, Toll-like receptor and DC-SIGN of DC, it is possible to select putative B- and T cell epitopes of *M. gallisepticum* and defined cytadhensins and other antigen components stimulating cellular and humoral immune response. These components can be combined with proper carrying system as well as with various adjuvant molecular patterns stimulating preferably Th1 response. To demonstrated this possibility we have performed some challenge experiments. In case of administrating antigens alone, we could produce protection by 52.8% or 64.3% in respect of decreased of lesions scores and resolution of mycoplasmas. However using antigen + adjuvant molecules, efficacy was reached up to 75.0% and 86.7 %. This approach allows to select such molecules which are not important in protection, to used them for developing differentiating ELISA tests and testing of vaccine candidates in vitro on dendritic cells.