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MYCOPLASMOSIS - A SERIOUS PROBLEM OF POULTRY INDUSTRY

Mycoplasmosis, commonly known as **chronic respiratory disease of chickens**, has existed in our country since time immemorial and was largely controllable. Recently, however, it appears to have emerged with renewed vigour, has become more common and a serious challenge to the industry. Why?

But before we examine this vital aspect of “Why?”, let us have a quick look at what mycoplasmas are?

Cause

The disease is caused by a **mycoplasma** known as *Mycoplasma gallisepticum* (MG). **The strains of MG may differ markedly in virulence, that is, in the degree of their disease-producing power. Some strains of MG are mild while others are highly virulent (very harmful).** Mycoplasma is similar to bacteria, but lacks a cell wall. Lack of wall makes MG extremely fragile. They are easily killed by disinfectants, heat, and sunlight. They remain alive in the environment, outside the bird, for only up to 3 days. For this reason, MG is fairly easy to eliminate on single-age, all-in all-out poultry farms.

Spread

M. gallisepticum **enters through the respiratory tract and/or eye.** It is transmitted through direct contact of infected birds, or through the environment, dust, soil, drinking water, and feed. It spreads only short distances by air (horizontal transmission). Once a chicken is infected, **the infection is of long duration.** The organism is present in the respiratory tissues in high levels and is shed into the environment and eggs. After several weeks, the level of infection and shedding of the organism decreases. But even then the infection persists in the flock indefinitely and the chickens may shed the organism periodically, especially following a period of stress. This makes elimination of MG extremely difficult in multi-age breeder and laying complexes.

The disease is spread from farm to farm mainly by **movement of contaminated people, equipment, and vehicles.** Thus **basic biosecurity** is the best means of preventing introduction of MG into layer and breeders complexes. **Because once infected, birds remain carriers for life.**

Egg transmission (vertical transmission) to broiler progeny occurs at a low level from infected breeders. However, horizontal infection then readily occurs in broiler houses. The organism is also spread by **wild birds.** Wild birds may become infected and shed MG.

The Disease Process

After entering the respiratory tract, the organism attaches to the lining epithelial cells. It secretes hydrogen peroxide which causes oxidative stress on the cell membrane. This facilitates its penetration. **Penetration of *M. gallisepticum* into cells occurs within 5 minutes** and the number of intracellular mycoplasmas (i.e., inside the cells) increases in 24 hours. Spread to other organs, for example, brain, indicates that brief systemic (blood) infections occur. Infection to oviduct may result because of its closeness to infected abdominal air sacs.

Although always considered as a pathogen (disease-producing organism) of the mucosal surface of the respiratory tract, **MG is able to penetrate cells. Once inside the cells, it easily avoids the action of antibodies and some antibiotics.** This, in turn, enables the organism to pass through the respiratory mucosal barrier and cause systemic infection. **Thus,** the cell invasion plays a major role in the systemic spread of *M. gallisepticum* and in escaping from the host defences. This, in turn, allows its survival in the bird and persistence of infection, hence the name **chronic respiratory disease (CRD).**

Why is *M. gallisepticum* a serious challenge to commercial poultry?

There are **four basic reasons** :

1. Our country witnessed the arrival of **Avian Influenza** in February 2006. Because of the stringent measures taken by the Government of India, the **highly pathogenic form of the disease (HPAI)** was brought under control but the **low pathogenic form of the virus (LPAI)** kept circulating, became endemic, and got itself firmly established as a permanent resident. Not only that, but during the six intervening years, it sharpened its razor and increased its disease-producing power (virulence). This happened because of the constant point mutations that occurred in its genes encoding haemagglutinin and/or neuraminidase surface glycoproteins (**antigenic drift**).

Antigenic drift gradually changes the structure of haemagglutinins and neuraminidases **resulting in increased disease-producing power.** As a result, infections by **drift variants of LPAI** in the field have become widespread and very harmful. **More recently, co-infection of *M. gallisepticum* and LPAI virus ((H3N8) in chickens has been reported.** In the light of this, it is suggested that LPAI may predispose the birds to *M. gallisepticum* infection and vice versa. That is, infection with *M. gallisepticum* may predispose the bird to the variant form of LPAI. This may account for the increased occurrence and increased mortality associated with *M. gallisepticum* nowadays.

2. An important feature of *M. gallisepticum* is its **antigenic variability.** To put it simply, it keeps changing its surface proteins (antigens). Proteins constitute more than two-thirds of the mycoplasma membrane, the rest being membrane lipids. The plasma membrane of *M. gallisepticum* contains approximately 200

polypeptides which are associated with surface antigenic variation. These antigens have been identified. They play key roles in the development of the disease (pathogenesis) and immune responses. *M. gallisepticum* is highly committed to antigenic variation and changes in the expression of the surface proteins. This leads to the production of “atypical” or “variant” strains of *M. gallisepticum*. As a result, the immunity produced by the administered vaccines is unable to deal effectively with the “variant” form of *M. gallisepticum*, and the infection may persist despite vaccination. Thus, antigenic variation is one of the simplest strategies adopted by this organism to avoid destruction by the immune defences of the bird.

3. Another characteristic feature of *M. gallisepticum* is **phenotypic switching**. To put it simply it is a very complex way in which this organism changes its surface antigens not only while outside the birds **but also while inside the bird**. These changes allow the organism to avoid the immune response of the bird, and therefore it persists in the bird for a longer period. *M. gallisepticum* has an inherent mechanism of making rapid and sudden changes in its expression of proteins in response to antibodies. Thus, this organism uses phenotypic switching for its survival in the bird. In other words, *M. gallisepticum* may **persist in the bird even in the presence of systemic or local antibody (carrier state)**.
4. Yet another important factor in the control of mycoplasmosis is the complex immune mechanisms involved in protecting the bird following vaccination. For example, there is poor correlation between the levels of circulating antibody and protection of the bird. It has been found that antibody in the respiratory secretions play a role in resistance against *M. gallisepticum*. **Respiratory tract antibodies inhibit attachment of the organism to tracheal epithelial cells, which is one important mechanism of protection**. It has now been established that although local immunity plays a main role in controlling *M. gallisepticum* infection, cell-mediated immune system is also involved. There is significant involvement of natural killer and cytotoxic-T cell responses.

To sum up, *M. gallisepticum* infection is a serious challenge because antigenic variation and phenotypic switching enable the organism to escape from the immune defences of the bird. This enables it to establish a chronic infection despite a strong immune response. Moreover, *M. gallisepticum* may hide from bird's defences by entering into the cells. Since antibodies and antibiotics cannot penetrate the cells, this strategy helps in its survival and persistence of the infection.

Incubation Period

The incubation period varies from 6-21 days. However, development of clinical signs can be highly variable depending on *M. gallisepticum* strain virulence (disease-producing power), complicating infections, and environmental

and other stress factors. Thus, many variable factors influence the onset and extent of clinical disease. **Therefore, meaningful incubation periods cannot be stated.** Layer birds usually develop clinical infections at the onset of egg production.

Clinical Signs

The most characteristic signs are tracheal rales (abnormal respiratory sounds), nasal discharge, and coughing. Feed consumption is reduced, and the birds lose weight. In laying flocks, egg production drops. However, flocks may show serological evidence of infection without clinical signs. Male birds may show more prominent signs, and the disease is more severe during winter. In broiler flocks, most outbreaks occur after four weeks of age. Signs are usually more marked than those observed in mature birds. Severe outbreaks with high morbidity (birds affected) and mortality, seen in broilers, are usually due to concurrent infections are usually due to concurrent infections and environmental factors.

Morbidity and Mortality

Although *M. gallisepticum* is the main cause of chronic respiratory disease, other organisms usually cause complications. Severe air sac infections, usually called **“complicated CRD (CCRD)”** or **“air sac disease”** is the condition more commonly observed in the field. Newcastle disease (Ranikhet disease), infectious bronchitis, or even LPAI virus may precipitate outbreaks of *M. gallisepticum* infection. *E. coli* is the most common complicating organism. The effects of *M. gallisepticum*, *E. coli*, and infectious bronchitis (IB) virus infections alone or together have been reported. As mentioned earlier, **recently co-infection of *M. gallisepticum* and LPAI virus ((H3N8) in chickens has also been reported.**

Mortality may be negligible in adult laying flocks, but there can be reduction in egg production. In broilers, mortality may vary from low in uncomplicated disease to severe in complicated outbreaks. Poor growth adds to further losses.

Postmortem Lesions

Lesions may not be visible or consist mainly of inflammatory exudate in nasal and paranasal passages, trachea, bronchi, and air sacs. In severe cases, there is **airsacculitis and air sacs usually contain cheese-like material (caseous exudate) resulting in high mortality.** These lesions are mainly because of the complication caused by *E. coli* infection. Oviducts distended with exudates (salpingitis) have been associated with decreased egg production in *M. gallisepticum*-infected flocks.

Immunity

Recovered birds have some degree of immunity. **However, recovered birds may still carry the organism and can transmit infections to susceptible birds by contact or egg transmission.** As already mentioned, there is poor correlation between the levels of circulating antibody and protection. Antibodies in the respiratory secretions play a role in resistance against *M. gallisepticum*. They inhibit attachment of the organism to tracheal epithelial cells, which is one important mechanism of immune-mediated protection. It has now been established that although local immunity plays a main role in controlling *M. gallisepticum* infection, there is also significant involvement of natural killer and cytotoxic-T cell responses in mycoplasma infection

However, changes in *M. gallisepticum* surface proteins which result in antigenic variation and phenotypic switching (discussed above), enable the organism to escape from the host immune defences (immune evasion) and **establish a chronic infection despite a strong immune response.** Moreover, the organism may hide from bird's defences by entering into cells (cell invasion) and spread systemically **which help in its survival and persistence of infection.**

Diagnosis

The standard method for *M. gallisepticum* diagnosis is isolation and identification of the organism. Serological procedures are useful for flock monitoring of *M. gallisepticum* control programmes. Haemagglutination-inhibition (HI) and ELISA tests have been commonly used to confirm reactors. Polymerase chain reaction (PCR) is used for rapid diagnosis. **A positive serological test along with history, clinical signs and postmortem lesions allow a presumptive diagnosis, pending isolation and/or identification of the organism.**

Low pathogenic form of avian influenza, Newcastle disease, infectious bronchitis, infectious laryngotracheitis, infectious coryza and other respiratory pathogens should be considered in the **differential diagnosis.**

Treatment

In the field, many cases of *M. gallisepticum* infection are complicated by other disease-producing bacteria. Therefore, effective treatment must also attack the secondary invaders.

Most strains of mycoplasma *M. gallisepticum* are sensitive to a number of antibiotics, but are resistant to penicillins or other antibiotics which act by inhibiting cell wall biosynthesis. *M. gallisepticum* may develop resistance to commonly used antibiotics. Antibiotics are usually given in feed or water. **A combination of colistin, tylosin, ciprofloxacin, and bromhexidine (a bronchodilator) in drinking water at the dosage of 1ml/2 litres of water for 3-5 days has been found effective. In case of severe**

infections, amikacin and tylosin combination each as 15 mg/kg body weight can be given as injection. This is very helpful.

However, complete elimination of *M. gallisepticum* from all birds in an infected flock by antibiotic treatment is an unrealistic hope. The treatment should be regarded as a method for short-term control of disease and economic losses, rather than as a long-term solution to the problem.

Prevention and Control

The continued presence and high incidence of MG in commercial poultry indicates that earlier efforts at its eradication were not very successful.

Therefore, a three-prong vigorous attack is required to deal with the problem of mycoplasmosis. It is extremely important that all the three measures are applied simultaneously. This is because each one has its own advantages and limitations, as explained below. However, if applied together, they bear fruit.

- 1. Use of antibiotics:** For example antibiotics such as kitamycin and colistin can be given in the feed regularly as antibiotic growth promoters. They are very helpful in the prevention of CRD.

Limitation: The antibiotics will have no effect on the organisms present inside the cells, and therefore complete elimination of *M. gallisepticum* in an infected flock cannot be achieved. **The organisms present inside the cells escape the action of antibiotics;** birds become carriers of infection and continue to shed the organisms. This results in contamination of the environment and spread of infection.

- 2. Use of vaccines:**

(a) Killed vaccines: *M. gallisepticum* killed vaccines (bacterins) protect young birds from infection with virulent *M. gallisepticum* and commercial egg layers from *M. gallisepticum*-induced drops in egg production.

Limitation: Antibodies will have no effect on the organisms present within the cells. However, killed vaccines have been shown to reduce but not eliminate colonization by *M. gallisepticum* following infection. They are thought to be of low value in long-term control of infection on multiple age production sites. Regarding effect of killed vaccines on the spread of the organism, there is some reduction in shedding, but vaccination does not reduce horizontal spread of *M. gallisepticum* between laying hens.

(b) Live vaccines: These are of three types: **F strain vaccine, 6/85 strain vaccine, and ts-11 vaccine.** Because of their greater safety (relative avirulence and low potential for transmission to unvaccinated flocks), **both 6/85 and ts-11**

vaccines may be preferred than F strain when *M. gallisepticum* vaccination is necessary.

Limitation: Because they are live vaccines, **there are concerns for the safety of F, 6/85, and ts-11 strains.** Even though these vaccines are generally very safe, **they may have the potential for infecting unvaccinated flocks.** Live vaccines should be used very carefully and administered following strictly the manufacturer's instructions and with careful consideration for the safety of unvaccinated flocks.

- 3. Management:** Because *M. gallisepticum* can be transmitted by egg, maintaining chicken flocks free of *M. gallisepticum* is possible only by starting with breeding stocks that are free of the infection. Then, they should be reared with adequate biosecurity to avoid introduction of the organism.

To sum up, each measure has its limitations. Moreover, because of overcrowding and lapses in biosecurity, maintaining *M. gallisepticum*-free poultry flocks may be difficult or impossible. **It is therefore of utmost importance that all the three measures, namely, administration of antibiotics, proper vaccination, and good management must be exercised together with full force to keep mycoplasmosis under control.**

Authors's Suggestions and Comments

- In *M. gallisepticum* infection, there is poor correlation between the levels of circulating antibody and protection. **Therefore, before killed vaccines are given, the birds must be primed with a live vaccine to ensure production of local immunity in the respiratory tract.** As already discussed, local immunity gives more protection than systemic immunity of killed vaccines. Killed vaccines do not produce effective local immunity. Repeating killed vaccines twice or thrice may not be helpful. This is particularly important for hatchery.
- **Antibiotics should be changed periodically** to avoid development of resistance.
- **Stringent biosecurity is extremely important in the control of disease for two reasons. Firstly,** since *M. gallisepticum* spreads only short distances by air, where excellent biosecurity is practised, there have been many cases when infection has not spread to neighbouring houses within a complex. **Secondly,** biosecurity would prevent any infection spread by the vaccinated birds.
- **Good management** by ensuring good ventilation, no overcrowding, and good nutrition are very helpful.
- **Finally,** it is strongly emphasized that *M. gallisepticum* must be controlled at all cost. If this is not done, apart from inflicting the damage on its own, **the disease**

will also act as a great predisposition to *E. coli*, Newcastle disease, infectious bronchitis, and possibly also to drift variant of LPAI.

- **To conclude, it is unlikely that MG will be eradicated from the commercial poultry industry in the coming years. However, though biosecurity programmes and effective use of vaccines, losses can be reduced.**
