Mycoplasma synoviae and Reovirus: (re)emerging infectious diseases in broiler Breeders

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ABSTRACT. Broiler breeders are one of the most important components of the poultry industry. This type of birds is susceptible to several agents that interfere with the immune system and predispose to infection. If transmission of pathogens to progeny is considered, their economic impact will be amplified in the broiler farms, compromising the entire production results. Construction of multi-age farms poses a significant epidemiological risk. In fact, these farms have grown in size and density, and an ideal environment has been created for agents such as *Mycoplasma synoviae* and *Reovirus* to thrive. A general review of the scientific literature concerning *M. synoviae* and *Reovirus* in broiler breeders is presented on their epidemiology, economic importance, pathogenesis, lesions, clinical signs, diagnosis, control, treatment and prevention.

**Keywords:** Broiler breeder; Infectious diseases; *Mycoplasma synoviae*; *Reovirus*
INTRODUCTION

Broiler breeders stay long periods in the rearing and production sites. This means that they are susceptible to several agents that interfere with the defense system and predispose to infection. Infections are very often apparently subclinical, but still induce damage in the infected hosts and may cause immunosuppression (Feberwee et al., 2008). If transmission of these pathogens to progeny is considered, the economic impact will be amplified to the broiler farms, compromising the overall production results (Kleven, 2003; Stipkovits et al., 2011). The increasing construction of multi-age farms (farms with birds of different ages) (Figure 1) poses a significant epidemiological risk. Although very strict hygiene rules are being implemented, poultry farms built in the latest years are designed mainly based on an economical perspective. Very rarely is disease prevention a primary consideration. The consequence is that farms have grown in size and density, and an ideal environment was created for agents such as Mycoplasma synoviae and Reovirus to thrive (Marois et al., 2005).

It is necessary to determine new and more effective strategies to reduce losses due to these and other agents (Kleven, 2003). Economic losses increase every day, including leg pathology reports, day-old chick quality decreases and slaughter condemnations (Catania et al., 2010; Landman and Feberwee, 2001).

Mycoplasma synoviae in broiler breeders

Mycoplasma spp. are widespread in nature as pathogens or commensals of eukaryotic hosts. These organisms are very small prokaryotes devoid of cell walls and bounded by a plasma membrane only (Kleven, 2003; Vogl et al., 2008). They were first isolated from chickens in 1935 (Nelson, 1936) and were later identified as avian mycoplasmas. Infections with M. synoviae have been reported in recent years as endemic in the poultry industry of many countries worldwide, where they cause considerable economic losses to heavy breeders, broilers and layers. As a testimony to the Mycoplasma’s resilience and adaptability, they continue to cause considerable economic losses to the poultry industry. The failure to eradicate M. synoviae from commercial poultry flocks has been largely due to the ability of these organisms to establish lifelong infections and to spread by horizontal and vertical transmission among their hosts (McAuliffe et al., 2006). The success of this fragile organism in infecting poultry flocks throughout the world indicates that evolving poultry management practices have facilitated the survival and transmission of this agent (Ferguson-Noel and Noormohammadi, 2013).

Aetiology and Economic importance

Several species of the genus Mycoplasmatare pathogens of mammals, birds, reptiles, fish and arthropods, causing a wide variety of diseases and having a predilection for the respiratory and the genital tracts as well as to joints (Vogl et al., 2008). Mycoplasma synoviae is a species of the class Mollicutes and was...
typically result in minimal losses; whereas an infection during egg production, especially during the peak of production, will cause a dramatic decline in egg production. In most cases, the egg production will recover but remain below the standard curve (Stipkovits and Kempf, 1996). The clinical and economic relevance of *M. synoviae* seems to be increasing considering the number of publications worldwide and the emergence of strains affecting eggshell quality (Figure 3) and egg production, and the emergence of arthropatic and amyloidogenic strains in some countries (Landman, 2014). 

**Epidemiology and pathogenesis**

*Mycoplasma synoviae* can be found in eggs laid by infected breeders. Vertical (i.e. transovarial) transmission is not very efficient, as peak egg transmission from an infected breeder flock is low. If complicating factors are present, such as immune suppression, there may be a higher shed of the organism (Behbahan et al., 2005). Vertical transmission plays a major role in spreading of *M. synoviae*. When commercial breeder flocks become infected during egg production, egg-transmission appears to be higher in the first 6 weeks after infection. After the chicks are hatched, *M. synoviae* organisms are spread horizontally. Transmission occurs among birds by the aerosol route and by contamination of the feed and water. The entire flock may be infected at 3 weeks of age (Kleven, 2003). Horizontal transmission readily occurs by direct contact. In general, *M. synoviae* appears to spread more rapidly than *M. gallisepticum*. The former can be present in the respiratory tract of infected chickens for 4 weeks and during that time the spread between houses occurs (Ferguson-Noel and Noormohammadi, 2013). Natural infection can be observed from the first week of age, but acute infection is more often seen when chickens are adult. This fact suggests that the incubation period can be relatively short, but it generally lasts for 11-21 days. Chronic infection may or may not follow the acute phase at any age and persist for the entire life of the flock (Kleven, 2003). Therefore, *M. synoviae* contaminated environment is a potential hazard to birds. *Mycoplasma synoviae* is also well known for its interactions with other infectious agents and environmental factors alike in producing clinical disease. Control of clinical manifestations is simplified when concurrent infections are minimized and optimal environmental conditions are provided. Respiratory infections are considerably
affected by environmental factors and disease severity is increased during the winter months. Temperature, ventilation, humidity, atmospheric ammonia and dust all have important interactions with infectious agents in producing respiratory disease (Landman, 2014). Atmospheric dust significantly increased the severity of air sac lesions, and chickens maintained at environmental temperatures of 7-10°C were more susceptible to airsacculitis caused by *M. synoviae* than chickens maintained at 24-29°C (Kleven, 2003). However, there have been relatively few studies on the influence of environmental factors on the severity of mycoplasma infections (Moreira et al., 2015b). Mycoplasmas also infect other domestic and wild avian species, so it is important to ensure they are not in contact with commercial chickens. Some data provide strong evidence that indirect transmission of *Mycoplasma* spp. via contaminated feeders occurs (Feberwee et al., 2005). Although *M. synoviae* can be transmitted via fomites, birds infected this way can quickly overcome mild disease and may on recovery be protected against more virulent infections acquired by direct bird-to-bird contact (Bebbahah et al., 2005). Such indirect transmission is rather unexpected for wall-less bacteria, which are supposed to be sensitive to osmotic shock, heating or chemical treatments. However, *M. synoviae* may persist on feathers up to 2 or 3 days at room temperature and its high dissemination capacity has been demonstrated (Marois et al., 2005). Mycoplasmas are more likely to spread among farms by the mechanical route, which includes spread via contaminated equipment, shoes and other fomites (Kleven, 2003).

*Mycoplasma synoviae* most frequently occurs as a subclinical upper respiratory infection. It may cause air sac lesions when combined with other respiratory agents such as Newcastle disease virus (NDV), infectious bronchitis virus (IBV), or both (Landman, 2014). Other times, *M. synoviae* becomes systemic and results in infectious synovitis, an acute to chronic infectious disease of chickens and turkeys, primarily involving joint synovial membranes and tendon sheaths, and producing exudative synovitis, tenovaginitis, or bursitis (Ferguson-Noel and Noormohammadi, 2013). Infectious sinusitis grossly distends infraorbital sinuses, with fibrin, heterophilic, epithelial cell hyperplasia, and hypertrophy of mucous glands. Later, there is lymphocytic infiltrates in the lamina propria or nodular formation, and tracheitis and airsacculitis can occur (Kleven, 2003). The pathogenicity of *M. synoviae* generally involves attachment and colonization of the respiratory tract, and other additional factors like immunosuppression can produce systemic invasion and clinical signs.

**Clinical signs and lesions**

Common disease signs like pale comb, lameness and retarded growth are the first noticeable manifestations. Disease progression debilitates the bird that became ruffled, and swellings usually occur around joints, especially the hock and foot pads joints (Ferguson-Noel and Noormohammadi, 2013). Airsacculites may occur in chickens infected via respiratory tract at any age. In recent years, the occurrence of arthropathic and amyloidogenic strains of *M. synoviae*, as well as strains that induce eggshell apex abnormalities and egg production losses, has increased (Feberwee et al., 2008). The progeny of *M. synoviae* infected breeders may have increased condemnation, poor conversion rates and poor weight gain. Morbidity varies from 2 to 75%, usually reaching 5 to 15%, and mortality ranges between 1 and 10%. As *M. synoviae* infection progresses, caseous exudates involve tendon sheats and joints that became thinned over time and may evolve into the muscle and air sacs. In the respiratory form, airsacculitis may be seen (Kleven, 2003).

**Diagnosis, control, treatment and prevention**

Diagnosis is based on epidemiological data, clinical signs, and analysis of macroscopic lesions, specific serology, isolation and molecular characterization of *M. synoviae*. Monitoring must be part of control programs performed in breeder flocks and is mostly feasible by routine serology and PCR (Kleven, 2003). Serologic procedures are useful for flock monitoring in *M. synoviae* control programs and to aid in diagnosis when infection is suspected. A positive serologic test, together with history and signs typical of the disease, allows a presumptive diagnosis pending isolation and identification of the organisms (Ferguson-Noel et al., 2011). The tube agglutination test was a common procedure, especially during the *M. gallisepticum* control program for turkeys in the 1960s and 70s but is now rarely used. Serum plate agglutination (SPA) antigen for the detection of antibodies to *M. synoviae* is commercially available. Because the SPA test is quick, relatively inexpensive and sensitive, it has been widely used as an initial screening test for flock monitoring and sero-
diagnosis. However, nonspecific reactors occur in some flocks infected with *M. synoviae* due to cross-reactive antigens, or those recently vaccinated with oil-emulsion vaccines and/or vaccines of tissue-culture origin against various agents. The SPA test is highly efficient in detecting IgM antibodies, which are the first class of immunoglobulins produced in response to infection (Kleven, 2003). The hemagglutination inhibition (HI) test has been commonly used to confirm reactors detected by SPA or, more recently, enzyme-linked immunosorbent assays (ELISA). However, the HI test is time consuming, the reagents are not commercially available and the test may lack adequate sensitivity. ELISA assays were developed to increase testing efficiency and improve sensitivity and specificity of results compared to the SPA and HI tests. Commercial ELISA test kits are now commonly used for serodiagnosis and flock monitoring. In general, ELISA tests are slightly less sensitive but more specific than SPA tests; and less specific but more sensitive than HI tests (Kleven, 2003; Ferguson-Noel and Noormohammadi, 2013). Ewing et al. (1998) reported that the SPA test missed infected commercial layer and breeder flocks that were detected by ELISA. Further confirmation of serologic results may be made by isolation and identification of *M. synoviae* from the upper respiratory tract or by PCR (Carli and Eyigor, 2002; Ramirez et al., 2006). However, few laboratories are equipped for culturing this organism, as specific culture media are required. Techniques for the detection and analysis of DNA through PCR arise as a very interesting alternative diagnostic method, because they offer sensitivity, specificity, capability of performing exams on a large scale and economic viability nowadays (Hammond et al., 2011). The sensitivity observed in PCR is important for detection of pathogenic agents in clinical samples taken from subclinically infected animals or those undergoing antibiotics treatment. Furthermore, it is possible to detect a pathogenic agent even before the host’s immunologic response, or in hosts with immunedepression, which points out advantages over the serologic tests (Buim et al., 2009; Kempf, 1998).

The antibiotic treatment of breeders is not effective for the elimination of *M. synoviae*, although egg transmission level is reduced (Kleven, 2003). Macrolides like tylosin and tilimicosin and fluoroquinolones like enrofloxacin and difloxacin are among the antibiotic families most widely used in poultry in many countries (Gerchman et al., 2011), but *M. synoviae* is susceptible *in vitro* to several other antibiotics including chlorotetra-cycline, lincomycin, oxytetracycline, spectinomycine, tetracycline and tiamulin (Ferguson-Noel and Noormohammadi, 2013) (Table 1). In the past, mycoplasmas eradication programs were based on antibiotic or heat treatment of fertile eggs, but more recently the intensive poultry industry relies heavily upon the application of vaccines for disease control (Ferguson-Noel et al., 2012). Vaccination programs are presently being used to control outbreaks of the more virulent strains of *M. synoviae* (Ferguson-Noel and Noormohammadi, 2013). Regarding the presence of mycoplasmas in breeder

### Table 1. Antimicrobial agents for treatment of mycoplasmosis in poultry.

<table>
<thead>
<tr>
<th>Mycoplasmosis</th>
<th>Type of activity</th>
<th>Side effects / recomendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st line-drugs</strong></td>
<td>Tiamulin</td>
<td>Bacteriostatic</td>
</tr>
<tr>
<td><strong>2nd line-drugs</strong></td>
<td>Tetracyclines</td>
<td>Bacteriostatic</td>
</tr>
<tr>
<td></td>
<td>Lincomycin</td>
<td>Bacteriostatic</td>
</tr>
<tr>
<td></td>
<td>Macrolides</td>
<td>Bacteriostatic</td>
</tr>
<tr>
<td><strong>3rd line-drugs</strong></td>
<td>Enrofloxacin</td>
<td>Bactericidal</td>
</tr>
</tbody>
</table>
also associated with a variety of problems including viral arthritis/tenosynovitis, enteric disease and malabsorption syndrome (Jones, 2013).

Aetiology and economic importance

Reoviruses have a worldwide distribution in chickens but are more related to meat-type birds (Van der Heide, 1977). They are commonly found in the digestive and respiratory tracts of clinically normal chickens and turkeys. It is estimated that most of the reoviruses isolated from chickens are non-pathogenic. Several studies performed over the last years have revealed unique properties for ARV e, different from those displayed by mammal viruses (Jones, 2013).

ARV, which replicate in the cytoplasm, are non-enveloped with an icosahedral symmetry and a double-shelled capsid and are one of the few non-enveloped viruses that cause cell to fuse (Xu and Coombsa, 2009). This specific genome segments responsible for protein coding have been identified for the S1133 strain of ARV and differentiates them phylogenetically from most other animal reoviruses (Day, 2009). Another interest characteristic of the ARV is that they are known to induce apoptosis in infected cells (Benavente and Martinez-Costas, 2007).

Avian reovirus infections are of economic importance to the poultry industry (Savage and Jones, 2003). In meat-type chickens, economic losses are frequently associated with reovirus infections. Increased mortality, viral arthritis/tenosynovitis and a general lack of performance are among the observed problems (Jones, 2013). Breeder flocks that develop viral arthritis just prior to the onset of or during egg production may, in addition to lameness, be affected by increased mortality, decreased egg production, suboptimal hatchability/fertility and vertical transmission of the virus to progeny.

Infectious viral arthritis is currently the best defined and most readily diagnosed reovirus (Rosenberger, 2003).

Epidemiology and pathogenesis

Reoviruses can be classified using serologic procedures or grouped according to their virulence. There are five serotypes of reoviruses from 77 isolates from intestines, respiratory tract and synovial isolates (Day, 2003).
2009). They are antigenically similar viruses and demonstrate clear strain differences based on virulence and virus persistence. There are considerable cross neutralization between heterologous serotypes (Islam et al., 1988). The ARV genome consists of 10 segments of double-stranded RNA: three large (L1, L2, L3), three medium (M1, M2, M3) and four small (S1, S2, S3, S4) (Jones, 2013).

In general, ARV is associated with arthritis, but they have also been identified as the etiological agents of other diseases. Some examples are malabsorption syndrome conditions, pericarditis, myocarditis, hydropericardium, enteritis, hepatitis, bursal and thymic atrophy, osteopetrosis, and acute and chronic respiratory syndromes (Rosenberger, 2003). Although reoviruses have been found in many avian species, chickens and turkeys are the only recognized natural or experimental hosts for reovirus-induced arthritis (Pertilem et al., 1996). Other bird species from which reoviruses can be isolated are ducks, pigeons, geese and psittacine species (Watier, 2010).

Initially, the ARV replicates in the villi of the small intestine and in the bursa, and then spreads to other tissues. Generally, osmotic diarrhea appears due to villi blunting (Rosenberg, 2003b). When a bird is infected by reoviruses, these increase susceptibility to other infectious agents (Watier, 2010). This immunosuppression is due to lymphoid depletion and compromise of the immune system. Some authors report age-related resistance to reovirus-induced arthritis (Jones and Georgian, 1984; Olson and Kerr, 1966). Again, this age-associated susceptibility may be related to the inability of young birds to develop an effective immune response (Jones, 2013). The virus can be spread laterally (horizontal transmission) but vertical and egg-transmission are also possible (Robertson and Wilcox, 1986). ARV may be excreted from the intestinal or respiratory tracts for at least 10 days post-inoculation. This fact suggests fecal contamination as a primary source of contact (Jones, 2013). Viral persistence can last for long periods, special in the caecal tonsils and hock joints (Savage and Jones, 2003). Birds that are infected at a young age are potential sources of infection (Rosenberger, 2003). Whether or not the disease occurs following infection with ARV, the incubation period ranges from 1 to 11 days and is highly dependent upon the virus pathotype, age of the host and route of exposure (footpad inoculation, intramuscular, intravenous) (Jones, 2013). Very often, infections are unapparent and demonstrable only by serology or virus isolation (Jones, 2013).

The virus frequently locates in the flexor and extensor tendons of the pelvic limb and is commonly seen in young birds (1-2 months). Mortality is usually low, but morbidity can be as high as 100%. Avian reoviruses possess group-specific antigen and serotype-specific antigen. Host’s humoral immunity (neutralizing antibodies) can be detected 7-10 days following infection. The presence of neutralizing antibodies and its importance in establishing protection is not well-defined yet. Birds may become persistently infected in the presence of high levels of circulating antibodies. It is apparent, however, that maternal antibodies can afford a degree of protection to day-old chickens against naturally occurring and experimental challenges. From several studies, the suppression of T-cell-mediated immunity by cyclosporin A resulted in increased mortality in reovirus-infected birds, but the relative severity of tendon lesions was not altered. Antibody protection is related to serotype homogeneity, virulence, host age and antibody titer (Grande et al., 2002; Jones, 2013; Rosenberger, 2003). For cell mediated immunity, the CD8+ T-cells may play a role in pathogenesis and/or reovirus clearance in the small intestine. Some authors have shown that challenging viruses are controlled in the absence of actively produced antibodies in B-cell immunosuppressed chicks (Day, 2009). This suggests that cellular immunity may be sufficient for broiler protection (Jones, 2013).

Clinical signs and lesions

In an acute infection, lameness is generally present and some chickens are atrophied (Crespo and Shivaprasad, 2011). In chronic infection, lameness is even more pronounced, but the percentage of infected chickens is small. Lameness in this type of lesions is due to enlargement in the area of the gastrocnemius or digital flexor tendons. In general, the rupture of the gastrocnemius tendon is noticeable (Figure 4). The swelling of the digital flexor and metatarsal extensor tendons is the more pronounced macroscopic lesion. Swelling of the foot pad and hock joint is less frequent, being marked by the edema of the tarsal and metatarsal tendon. Some petechial hemorrhages are frequent in the
In more recent years, ELISA for detecting antibodies to avian reoviruses along with PCR has become more common (Bruhn et al., 2005).

The ubiquitous nature of the avian reoviruses and their inherent stability, coupled with modern, high-density confinement rearing practices, suggests that elimination of virus exposure may be difficult (Jones, 2013). Resistance to inactivation may be frequently carried by mechanical means like brooding temperatures. Commercially available disinfectants should be validated for efficacy before use, because of the avian reovirus group relative stability (Rosenberger, 2003).

Chickens are most susceptible to pathogenic reoviruses at 1 day of age and then develop an age-associated resistance from as early as 2 weeks (Kerr and Olson, 1964). Vaccines and vaccination programs have evolved and can provide protection at 1 day of age onwards. Active immunization can be achieved by vaccination with viable attenuated reoviruses, which are usually applied by the subcutaneous route (Giambrone and Clay, 1986).

Reovirus vaccination of breeding stock can be carried out with live attenuated or inactivated vaccines (Table 2). The latter are more effective when preceded by vaccination with a live vaccine. If a live vaccine is used, it should be administered prior to the onset of egg production, to prevent transovarian transmission of the vaccine virus (Jones, 2000). The advantages of this type of immunization program include immediate protection of the day-old progeny as provided by maternal antibodies (Jones, 2013). Vaccination of breeders is an effective method of controlling viral arthritis and other pathogenic reoviruses, but it should be recognized that protection is assured against homologous serotypes only (Rosenberger, 2003).

**CONCLUDING REMARKS**

The poultry industry is constantly under development, especially at the broiler breeder level. The reviewed infectious diseases are those which currently

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Table 2. List of vaccines approved in the European Union.

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Vaccine strain</th>
<th>Type</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avipro Reo</td>
<td>1133</td>
<td>Live</td>
<td>Lhomann-AH</td>
</tr>
<tr>
<td>Nobilis Reo 1133</td>
<td>1133</td>
<td>Live</td>
<td>MSD-AH</td>
</tr>
<tr>
<td>Nobilis Reo 2177</td>
<td>2177</td>
<td>Live</td>
<td>MSD-AH</td>
</tr>
<tr>
<td>Nobilis Reo inac</td>
<td>1733 and 2408</td>
<td>Inactivated</td>
<td>MSD-AH</td>
</tr>
</tbody>
</table>

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pose the greatest difficulties to the business operators, due to the lack of updated knowledge, the issues sensi-

bility, their indirect impacts (chick’s quality), and difficult resolution. At the broiler breeder level, the locomotor problems have been identified as an important emerging factor. *Mycoplasma synoviae* and *Reovirus* are the major infectious causes of their emergence. Another important related factor is the improvement of poultry genetic strains seen in recent years. Birds have now better performances which are reflected in their metabolism and structure. The industry itself has grown tremendously, with increased number of birds, multi-age farms and larger structures. The vertical transmission characteristic of these agents and chick’s quality is currently a major key point in the poultry business.

The economic impact of *Reovirus* in broiler breeders has been a subject of controversy. Nevertheless, economic losses may increase every day, including leg problems. This fact raises awareness among the poultry community all over the world. If vertical transmission of the agent is considered, costs to poultry producers might further increase.

Although very strict hygiene rules are being imple-

mented, poultry farms built in the latest 10 years have been designed to keep in mind an economical perspective. Disease prevention is very rarely a primary consideration in the modern poultry industry. A consequence is that farms have grown in size and density, and an optimal environment has been generated for agents such as *M. synoviae* to thrive.

Intervention strategies as management and bios-

security procedures are necessary to reduce economic losses due to *M. synoviae* and *Reovirus*. Parent stock should be free from *M. synoviae* and if this is not possible managers should trace specific positive breeder flock and their progeny should be hatched separately. Antibiotic medication is available but is not thought to eliminate *M. synoviae*. Reoviruses are very resistant to inactivation and may frequentlt be carried by mechanical means. Cleaning and disinfection practices should be taken into attention with special emphasis on iodine solutions. *M. synoviae* vaccines are commercially available, both inactivated or live vaccines, and appear to be safe and effective. Vaccinating the parent stock with *Reovirus* vaccines provide chicks with good levels of maternal antibodies and will minimize reoviruses from reaching the joints. Both inactivated vaccines (mainly 1133, 1733 and 2408 strains) and attenuated vaccines (1133 and 2177 common strains) are available for immunization of breeders and broilers, and this is an effective method of controlling viral arthritis.

Poultry industry will face important challenges in the coming years. The increase in world human population, the shortage of food and water, will put poultry in the top priorities in the livestock sector. Chick’s quality will represent an increasingly important issue over the years. Locomotion problems and the demand for high quality chicks will be ongoing challenges, and a focus on these issues should be prioritized. Only with quality chicks will be possible to achieve better performances in the present and in the future. Knowledge of the mentioned emerging problems in broiler breeders together with new strategic views will be key points to success.

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