

ABSTRACT

The importance of micoplasma induced diseases rises every day, because avian micoplasmosis are infections with a persistent chronic character, spread throughout the world. In poultry, the micoplasmic infections stay one of the most frequent causes of major economic losses, due to the drop of the productivity factors: daily growth, feedingstuff specific consumption, slaughtering age, egg laying percentage, hatching percentage, which require costly treatments to be resolved.

The Doctoral Thesis entitled „*Research regarding the immune response in respiratory avian Mycoplasmosis*” spreads throughout 192 pages and consists, in conformity with the actual legal provisions, of two main parts: the first part entitled „*The actual state of knowledge*” which is framed within 49 pages, 6 tables and 13 drawings, and the second part „*Personal contributions*” which contains 113 pages, 31 tables and 47 drawings for a better presentation of the contents.

The first part is made of four chapters, in which there are presented information from specialty literature regarding the subject of the thesis, that were later used to interpret and compare data obtained in the second part.

The first chapter, entitled „*Biographic data regarding the history, spread and importance of respiratory avian mycoplasmosis*”, presents data from specialty literature referring to the first descriptions of the disease and of the etiologic agent, the spread and importance of the disease. The respiratory avian mycoplasmosis known in the past as chronic respiratory disease (C.R.D.), is an infectious disease that affects poultry, turkeys, pheasants, guinea hens, quail, peacocks. It was described for the first time in the U.S.A. by Delaplane in 1943, the causing agent was identified as the microorganism in the PPLO group in 1952 by Markham and Wong. In our country the disease was diagnosed for the first time in 1958, after the import of poultry from Canada.

The second chapter, entitled „*Biographic data regarding the etiology, epidemiology and pathogenesis in respiratory avian Mycoplasmosis*” is structured in four subchapters, synthesising the main data from specialty literature referring to the nature of the etiological agent, the genomic organisation of *Mycoplasma gallisepticum*, its proteins and its enzymes,

describing each one's role, the receptors and the cellular targets of *Mycoplasma gallisepticum*, criterion and principles of taxonomic classification of the micoplasmas. In the second subchapter are presented the sources of infection, highlighting the manner of spread and the favourising factors for the apparition of the disease. Resistance to the physico-chemical factors. In subchapter three the pathogenesis of the disease is described, and in subchapter four the cellular and humoral immuno response to the mycoplasma infection and the factors that influence the antimicoplasmic immunity are presented.

Chapter three of the first part entitled „*Bibliographical data regarding clinical, morphopathological and hystopathological aspects in respiratory Avian Mycoplasmosis* ” treats aspects referring to the symptoms and lesions induced by infections with *Mycoplasma gallisepticum*.

The evolution and clinical manifestation are based on age, location of the inflammatory process and the secondary infections, in general complications with *Echerichia coli*, *Proteus spp.*, *Pseudomonas spp.*, *Haemophilus spp.*

In the case of the transmission of the virus in vertical, through the egg, the first signs of disease appear in the first day of hatching. The chicks present in serous jetaj initially, then seromucos, mucous, mucopurulent. Coughing and sneezing are present, also respiratory rattle in the trachea and air sacs. Breathing is done with the beak opened. Sinusitis appears with exudative character.

Beggining with the age of 25-30 days the clinical signs get complicated, the chicks sneeze, they cough, have sinusitis of different intensities, the diminished appetite, no liftitude, the stay more on one side, thus causing injuries on the sternum level, the tibia are reddened, inflamated and painful.

After the age of three months, when the disease cand appear only due to the aerogen transmtion or through direct contact, the symptoms decrease in intensity in comparison to the youth, the disease having a unpatterned evolution. In adult hens breeders broilers, the disease has chronic evolution. Rarely we meet head cold syndrome. In return, we register drops in the main indicators: egg production, hatching percentage, infant mortality, chick mortality. There can appear soft eggshells, small or deformed eggs. In return, adult hens breeders layers are carriers but do not manifest clinical symptoms, but are a important source of infection to breeder broilers.

The anatomopathological modifications are variable, in function of the age of the poultry and the evolution state of the disease. In the initial state of the disease we encounter sinusitis, sometimes laryngitis. To these we add the lesions on the windpipe. In the airsacs we find or mase fibrinous or caseous, in regular conglomerates of issues,"the egg greaves" that can block them

parially or totally. Likewise we can encounter caseous fibrin plugs that block the respiration, being usually at the trachea bifurcation.

In the advanced forms of the disease, especially when there is intervention from other germs also-most frequently *Escherichia coli* – we notice fibrin fibrinous or caseous inflammation of most serous of thoraco-abdominal cavity, particularly pericardial, epicardial, with fibrinous adhesions between the pericardium and epicardial, and airsaculitis perihepatite. For chicks, we mention the frequent presence of salpingitis pseudomembranous in which the fibrinous exudate is eliminated in the form of an pseudoconcrement in the begining of the egg laying. It is of a very great importance, marking the existance of the infection in the unit. Histological examination revealed hyperplasia of nasal glands, which emphasizes a hollow part which can be observed in the tracheal epithelium. Tracheal mucosa shows lymphoid infiltration and hyperplasia of mucous glands, that they take the shape of an amphora.

The Nobilis MG 6/85 vaccine induces a celular response. Thus, the immune sistem of the vaccinated birds does not produce antimicoplasma antibodyes. We can now tell the difference between a contaminated bird with antibodyes and a uncontaminated one. Studies have been done to see the immune response of broiler flocks derived from vaccinated parents, they will be presented in Chapter IX. After six consecutive vaccine series in 2003-2006 in which only the last three series vaccinated by aerosol from the age of 14 days, not six weeks (42 days) as the manufacturer recommends the vaccine, we obtained the desired result: the actual breeding heavy breeds - jelly and broiler flocks without Mycoplasma lesions and free of antimicoplasma-antibodyes.

Chapter VIII entitled ***„Research on the immune status in Avian Mycoplasmosis, before and after the vaccination with live Nobilis MG 6/85 vaccine ”*** presents on the course of three subchapters, the testing programs regarding the immune status in micoplasmosis and the results obtained in the serologic screening done in the reproduction farms in 11 series of parents broiler breeds, which were immunized in different ways as presented in chapter VII.

A rapid serum agglutination method was used on lama and immunoenzymatic ELISA test. On all the series tests were made on ages of a day, 14, 28-44, 70, 90-100, 130 and on adult hens on different ages. 10 blood samples/hall were taken, both from male and female. All the blood samples tested at the age of one and 14 days at a total of 2640 were negative. They did not have antimicoplasma antibodyes. Thus, the reproduction youth was not contaminated at this age.

At the age of 40 days, one day before the vaccination, there were taken and tested a batch of 360 samples, from the first 3 series (S1-S3) vaccinated in ocular, oculonazal, oculonazal-aerosol at 41 days age. At 57 of these there were detected antimicoplasma antibodyes,

representing 16% of the samples. These birds were positive at all the serologic tests until the closing of the egg-laying cycle. They transmitted the mycoplasma through the egg, and the broiler that came from this stock was infected, the results of the clinical, pathological, serological tests and the technological performances are presented in chapter IX and X. In the next 2 series (S4-S5), vaccinated only through the aerosols at the age of 14 days, the percent of positive birds dropped by 3%. Of 240 samples tested serologically 8 were positive. At 6-11 (S6-S11) series there weren't any antimycoplasma antibodies detected. All the 720 elisa tests made were negative. They maintained negative till the egg-laying cycle.

In chapter IX, entitled „*Research on the stability of the vaccine strain and the immune response of offspring obtained from Ross 308 heavy broiler breeds vaccinated with live Nobilis MG 6/85 vaccine*” we present in 3 subchapters, the research done to determine the stability of the vaccine strain Nobilis MG 6/85 and the results on the immune response of the offspring obtained in the vaccinated parents. For determination of the stability we vaccinated 36500 chicks with Nobilis MG 6/85. After the hens entered the laying period eggs were harvested and put into incubation.

Samples were taken from the embrionated eggs, from the internal surface of the yolk membrane, from the air sacs, the pharynx, the trachea, the tracheal tampons from the dead embryos remained in the shell and infant dead; exudate from aspiration of the sinuses, nasal cavity and the air sacs from the broiler chicks 20 days old also 50 blood samples for detecting antimycoplasma antibodies from one day old chicks. Samples were worked in the Pasteur, The Research Department-The molecular and serological biology laboratory Bucharest and in Laboratoire D'Analyses de biologie veterinaire et de L'Environnement-Bio Chene Vert France. Bacteriological examinations were done by growing on specific environments for isolating Mycoplasma gallisepticum-broth base PPLO and purified agar for mycoplasmas. The harvests were declared negative after 20 days from the seeding.

They were also examined with the PCR technique-method for bacteria DNA detection from tissues and secretions harvested for analysis presented earlier. The result is negative at all the tests. Elisa tests were also done for detecting antiMycoplasma antibodies in one day old chicks from the incubation station. The result was negative for all the 50 worked samples.

The conclusion after these tests is that the live vaccine strain Mg 6/85 is not transmitted vertically through the egg, it is a safe vaccine that can be applied without reserves also to the breeding broilers-not only in breeders layers and layer hybrids. The immune response of the offspring obtained from the vaccinated parents highlights the fact that the vaccination through

the aerosol of the parents, before the contamination with wild strains may lead in an interval of 2 to 3 years with respecting biosecurity measures, in flocks free of mycoplasma gallisepticum.

Chapter X entitled „ *Research on the health status and technical performances of offspring obtained from Ross 308 heavy broiler breed vaccinated against Avian Mycoplasmosis* ” contains information regarding results obtained in broilers. Broilers obtained from S6-S11 reached the target set by us: chicks without respiratory syndromes due to mycoplasmosis. Treatments against avian mycoplasmosis were removed from the broiler farms and from the laying farms.

For monitoring the pathological situation of the offspring there were 2950 necropsies done. The main lesions encountered were airsacculitis fibrinous - caseous stoppers fibrinous or fibrinous - caseous trachea and branch located in the bronchus, opacity of air bags, peritonitis. The percentage of loss through mortality was down from 12% the amount obtained in S1 to 2% at chicks obtained S6. The occurrence of specific lesions due to mycoplasmosis dropped from 65% in chicks S1 to 0 at chicks from S6.

The technical performances obtained are: reduced number of confiscated products in slaughter-house from 3,5% to 0; the hatching percentage in incubation stations improved by 4% rising from 84% to 88%, the costs on medication in the broiler were of 5% from the total expenses /live kg. After the sixth series vaccinated these are no longer done. Only the cost in vaccinating parents remains, which is 0,2% from the total expenses. The specific feedstuff consumption dropped from 2.025 kg /live kg , to 1.8 kg/live kg. The body weight of one day chicks is with 10-15 grams bigger than chicks that come from S1 (40-45 g compared to 30-32 g).

The financial situation was visibly improved, production cost /live kg dropping from 3,5 lei /live kg to 3,2 lei / live kg.

These results were obtained in the year 2007, after the sixth series of parents vaccinated with live Nobilis MG 6/85, representing the 3rd series of parents vaccinated consecutive through aerosol until the year 2009 in the month May when research was completed.

In the XIth chapter final conclusions are presented, in number 22, which were obtained after a long period of extensive research over 10 years.