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**I. Iancu<sup>1</sup>, N. Cătană<sup>1</sup>, V. Popa<sup>2</sup>, J. Degi<sup>1</sup>, C. Pascu<sup>1</sup>, V. Herman<sup>1</sup>, G. Ilgekbayeva<sup>3</sup>**<sup>1</sup>Banat's University of Agricultural Sciences and Veterinary Medicine;  
"King Michael I of Romania", Timisoara, Romania;<sup>2</sup>Pasteur Institute, Bucharest, Romania;<sup>3</sup>Kazakh National Agrarian University, Almaty, Kazakhstan.E-mail: ionicavet@gmail.com, epirovet.tn@yahoo.com, virgilia.popa@gmail.com,  
janos.degi@gmail.com, corina.pascu.ro@yahoo.co.uk, viorel.herman@fmvt.ro, gulnaz66@mail.ru**DETECTION OF MYCOPLASMA SYNOVIAE ANTIBODIES  
IN SERA OF BROILER CHICKENS BY THE ELISA**

**Abstract.** *Mycoplasma synoviae* (MS) infection most frequently occurs as a subclinical respiratory infection. Approximately 2-4 weeks are required for antibodies to develop in infected birds.

Clinical examination was made daily, in broilers after 10 days old, because at this age there were seen the first symptoms of disease.

Enzyme-linked immunosorbent assay is commonly used as a diagnostic test and for routine testing of flocks and may replace serum plate agglutination as the primary serologic test. The serologic exam carried out through ELISA test at 21 and 35 days evidenced the specific antibodies and sero-conversion phenomenon.

At 21 days old (B I), 22, 91% from the analysed sera were positive, and at 35 days old (B II), 26,66% were positive, until the age for slaughter (42 days).

The value of titers geometrical mean of 10,92 times higher at 35 days old than at 21 days old and the higher proportion of the obtained positive titers, reveal both the implication of *M. synoviae* in the etiology of disease, and her infectiousness, by horizontal transmission.

**Key words:** *Mycoplasma synoviae*, broiler chicken, ELISA.

**Introduction.** *M. synoviae* commonly causes inapparent respiratory infection but may result in airsacculitis and synovitis in chickens and turkeys. *M. synoviae* is responsible for avian infectious synovitis, a disease which occur to chicken and turkey, characterized by an inflammation of synovial membranes of joints and sinews, producing important economical losses by stopping the affected poultry growth [1,2].

Poultry serological monitoring should be performed periodically, whereas the cases suspected by an infection with *Mycoplasma* should be confirmed by agent isolation or by ELISA [1,2].

The existing data in the literature indicate that it is an infectious avian synovitis, the chickens, develop sporadically or endemic, the morbidity being between 2-75% and the mortality is 1-30%. Typically, in broiler chicken, the disease have synovial and respiratory locations. It is also possible to develop an evolution of respiratory mycoplasmosis that can have 80-90% morbidity and 30% mortality [3].

Symptoms appeared in chickens after 3 weeks of age. The sick chickens had reduced appetite, adynamy, difficult walking, stinging, uni- or bilateral arthritis in the tarsus-metatarsal joint. The joints were enlarged in volume, fluctuating and sensitive. For these reasons, the chicks refused to travel, to drink water and to feed, that is why the progressive weakening appeared. Some chickens, in addition to the locomotor symptoms, had dyspnea and tracheal rales.

Kleven and Fletcher observed the appearance of clinical signs, in the natural infection in the chickens, starting at the age of one week, although the acute infection usually occurs in the chicks between 4 and 16 weeks [4].

In the case of experimental infection with joint trituration, from chickens with clinical signs of disease, the incubation period ranged from 2 days, in the case of intravenous inoculation, to 21 days, in the case of aerosol infection [2,5].

In the specialized literature it is mentioned that the infection translates into an exudative synovitis, characterized in the first phases by an inflammation of the membranes of the synovial tissues in the region of the knees, phalanges, sternum and mandibles, externalized by the accumulation of an exudate with appearance. viscous, consistent, gray - yellow, which in the case of chronic infections turns into a chaotic mass [6].

Kerr and Olson revealed, in the experimental infections, the erosive character of the arthritis produced by *M. synoviae*: at 165 days post-infection the articular surfaces are completely destroyed and replaced by processes of fibrous organization, with adhesive tenosynovitis, which subsequently lead to joint ankylosis [7].

The presence of *M. synoviae* infection in the broiler chicken parents population was a source of infection for the day-old chicks resulting from them, as well as the presence of the adult poultry, in the same farm as the chickens, could be a reservoir of *M. synoviae* and *M. gallisepticum*.

Recording of increased levels of antibodies against *M. synoviae* and *M. gallisepticum* in chickens, at the age at which the outbreaks of disease evolved, confirms the existence of the post-infectious immune response.

**Materials and methods.** In this research were described an episode from the broilers farm in West of Roumanie, consisted of 2 sheds populated with broilers (the hybrid Ross 308), usually from import. In that case, there was suspected avian infectious synovitis.

For the confirmation of diagnosis, we have performed epidemiological, anatomic-pathological and serological examinations.

The epidemiological and anatomic-pathological exams were made in the farm and the laboratory exam was made in Infectious Diseases Department of FMV Timisoara and in S.N. Institutul Pasteur S.A. București.

In the epidemiological examination, the main parameter supervised was represented by cumulative mortality.

Clinical examination was made daily, in broilers after 10 days old, because at this age were seen the first symptoms of disease. We have not performed determinations concerning the microclimatic parameters.

Serological examination was made to confirm the suspicion of avian infectious synovitis. There were collected blood samples from broiler chickens, randomly, in the next way:

- B I – at 21 days old (48 blood samples);
- B II – at 35 days old (30 blood samples).

The serum samples were decanted in Ependorf tubes, counted off and kept in the deepfreeze, until the serological exam.

The sera were assayed for detection of specific antibody, using commercial ELISA (Enzyme Linked Immunosorbent Assay) kit, named Mycoplasma Synoviae Antibody Test Kit from Affinitech Ltd. [8].

The serological exam was realised in S.N. Institutul Pasteur S.A. București.

**Results and discussions.** Serological examination had as purpose the confirmation of avian infectious synovitis, epidemiological and anatomoclinical suspected, in the series of broiler chickens from the studied farm.

The results of epidemiological examination are expressed by the cumulative mortality recorded in broiler chickens was about 25%, up to the age of slaughter. These values correspond to the data from the literature, most of the authors considering that, in mycoplasmosis of broilers, the cumulative mortality exceeds 20% [6].

The results of anatomo-clinical examination showed that, after 4 weeks of age, in some chickens, the gastrocnemian tendon ruptured (figure 1), followed by the impossibility of displacement and extension of the affected limb. As a rule, this change was unilateral.

According to arthritis, in the early stage, the articular exudate in the age of gray-yellow, still during the chronic evolution, the exudate was caudal and contained a synovial tendon tissue.

Frequently, gastrocnemius tendon rupture and joint surface destruction have been reported.

In addition to the inflammatory lesions (figure 2) of the locomotory apparatus, hepatomegaly, splenomegaly and sternal bursitis have also been observed.

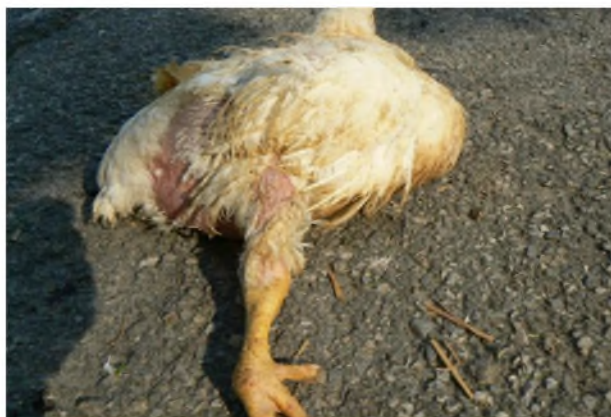


Figure 1 – Broiler chicken with unilateral tear of the gastrocnemius tendon



Figure 2 – Inflammation of the synovial sheaths

The results of this exam, made by ELISA test, are shown in table and figure 3. After the interpretation of reactions and the processing of results, according to the interpretation soft of the FlockChek<sup>®</sup> Avian MS Antibody Test Kit, there were assigned for every recoltation: the titre group, the minimum titre, the maximal titre and the geometrical mean (G.M.). The titers are expressed in optical densities (O.D.).

The results of serological exam performed by ELISA

No. crt.	B 1/21 days		B 2/35 days	
	Titre group	Samples number	Titre group	Samples number
1	0	37	0	22
2	1	4	1	5
3	2	4	2	1
4	3	2	3	2
5	4	1	4	0
7	Maximal titre	3000 O. D.	Maximal titre	2293 O. D.
8	Minimum titre	0 O. D.	Minimum titre	15 O. D.
9	Titers geometrical mean	13	Titers geometrical mean	142

In the first bleeding, at 21 days old, were identified 5 titers groups (0-4), minimum titre was of 0 O.D. and maximal titre was of 3000 O.D.

In the second bleeding, at 35 days, were identified 4 titrers groups (0-3), minimum titre was of 15 O.D. and maximal titre was of 2293 O.D.

From these results we observed that at 21 days old (B I), 22, 91% from the analysed sera were positive, and at 35 days old (B II), 26,66% were positive.

The results of serological exam showed that at 21 days of age the geometric mean of the titres had a value of 13 O.D. compared to the geometric mean titres at the age of 35 days, when it registered a significant increase, being 142 O.D.

At that age, the antibody titrers expressed in O.D. were much higher than the positive sera proportion: at 35 days old, was of 10,92 times higher than the titrers G.M. at 21 days old. That evolution of seroconversion suggests the aggressive character of mycoplasmas and their active implication in the pathological process.

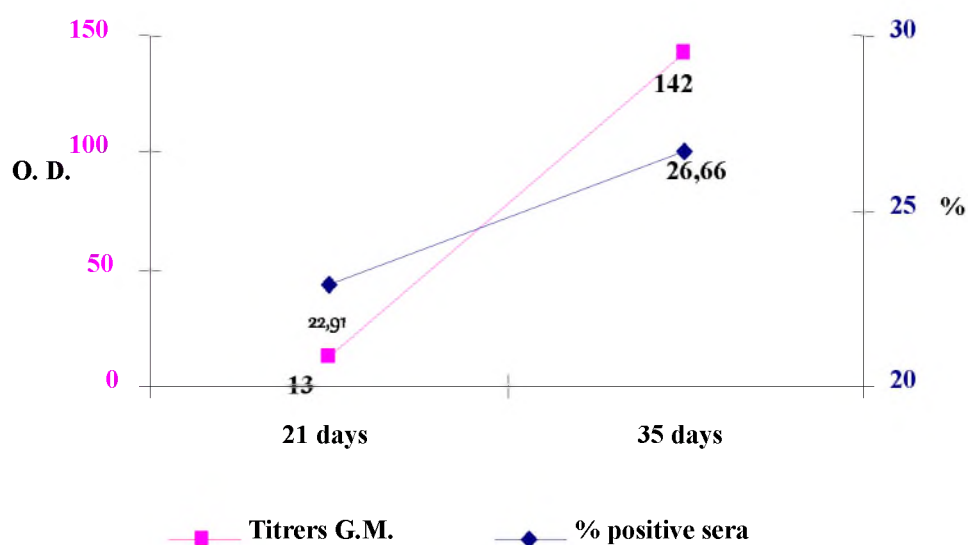


Figure 3 – Titers geometrical mean, expressed in O.D., and the proportion of positive sera

The results of serological exam confirmed the presence of *M. synoviae* infection, which was suspected by the epidemiological and anatomoclinical exams.

In the same time, the results of this research demonstrated a postinfection specific immune response.

The values and the proportion of positive titrers obtained at 21 and 35 days old reveal both the implication of *M. synoviae* in the etiology of the disease, and her infectiousness, by horizontal transmission.

The values of the antibodies anti *M. synoviae* titrers, which are expressed in O.D., are similar to the values noted by other authors [6,9].

Analysis of the results obtained found that broiler chickens showed a high level of antibodies against *M. synoviae*.

The results suggest a high prevalence of *M. synoviae* in chickens aged 35 days. This could be an alarming sign in countries where there is no control / vaccination program targeting *M. synoviae*. High levels of anti-*M. synoviae* antibodies recorded in broiler chickens suggest that they are exposed to the infectious agent.

In accordance with the kit manufacturer's recommendations, all the sera collected from broiler chickens, which had a titre value of less than 400, were considered negative.

In contrast, all serum samples from chickens were serologically positive for *M. synoviae*, with very high levels of antibodies and an average titer of 3000, assuming a severe infection with this pathogen, probably associated with some respiratory viruses.

Infection with *Mycoplasma synoviae* is a threat to the economic evolution of poultry, because infected birds are an active reservoir of *M. synoviae*.

Our results are in line with those published in the poultry units located in Western Europe, where an increase of *Mycoplasma synoviae* infections was observed, the isolated strains being resistant to erythromycin and flumequin and sensitive to oxytetracycline, doxycycline, spiramicin, lincomycin, tylosin thiamine, enrofloxacin and spectinomycin [10-15].

Serological monitoring of poultry is the correct measure for controlling infections with the so-called preventive treatments required where necessary.

Conclusion:

- There was signaled the existence of one disease with symptoms specific to the infectious mycoplasmosis, the form of avian infectious synovitis.

- The confirmation of disease was made by the ELISA test, using *Mycoplasma Synoviae Antibody Test kit*.

- At 21 days old (B I), 22, 91% from the analysed sera were positive, and at 35 days old (B II), 26,66% were positive, until the age for slaughter (42 days).

- The value of titers geometrical mean of 10,92 times higher at 35 days old than at 21 days old and the higher proportion of the obtained positive titers, reveal both the implication of *M. synoviae* in the etiology of disease, and her infectiousness, by horizontal transmission.

**И. Ианку<sup>1</sup>, Н. Катана<sup>1</sup>, В. Попа<sup>2</sup>, Ж. Деги<sup>1</sup>, К. Паску<sup>1</sup>, В. Херман<sup>1</sup>, Г. Ильгекбаева<sup>3</sup>**

<sup>1</sup>«Румыния Майкл I Король» Банат ауылшаруашылық ғылымы және ветеринариялық медицина университеті, Тимишоара, Румыния;

<sup>1</sup>Пастер университеті, Бухарест, Румыния;

<sup>3</sup>Қазақ ұлттық аграрлық университеті, Алматы, Қазақстан

#### **ИФТ-ДА БРОЙЛЕР БАЛАПАНДАРЫ ҚАН САРЫСУЫНДА MYCOPLASMA SYNOVIAE-ГЕ ҚАРСЫ АНТИДЕНЕНІ АНЫҚТАУ**

**Аннотация.** Құстардың жұқпалы синовит диагнозын растау үшін серологиялық зерттеу жүргізілді. Зерттелген шаруашылықта індеттанулық және анатомиялық-клиникалық деректер байынша бройлер-балапандар күдік тудырды.

Эпизоотологиялық зерттеу нәтижелері бройлер балапандардың өлім-жітім жиыны сойыс жасына дейін 25% - ға жуық екендігін көрсетті.

Анатомиялық-клиникалық зерттеулер кейбір тауықтардың төрт аптадан кейін балтыр сіңірінің үзілгенін, олардың алдағы уақытта орнынан жылжу және зақымдалған аяғын бүге алмайтынын айқындады.

Артрит аумағында, ерте кезеңде буын экссудаты сұр-сары түсті болғанда тіптен созылмалы эволюция кезінде қаудалды жалқаяқ болып, сіңірдің синовиалды ұлпасы анықталды.

Серологиялық реакция FlockChek® Avian MS Antibody Test Kit қойылып, оның нәтижесі бағдарламалық жасақтама арқылы өңделгеннен кейін әрбір талдау барысында келесі көрсеткіштер анықталды: топ титрі, минималды титр, максималды титр және орташа геометриялық титр. Титр оптикалық тығыздық негізінде (О.Т.) көрсетілді.

Бірінші зерттеуде 21 күндігінде титрдің 5 тобы (0-4) анықталып, минималды титр 0 О.Т. және максималды титр 3000 О.Т. болды. Екіншісінде 35 күннен кейін 4 титрлі топ (0-3) анықталды, минималды титр 15 О.Т. және максималды титр 2293 О.Т. болып анықтады.

21 күндіктерде (B I) талдау жасалғандардан 22,91% қан сарысуы оң нәтиже беріп, ал 35 күндіктерде (B II) тіпті сою уақытына дейін (42 күн) 26,66% оң нәтиже көрсетті.

Серологиялық зерттеулер нәтижесі титрлердің геометриялық орташа мәні 21 күндіктерде 35 күндіктерге қарағанда 13 О.Т. болды, ал 35 күндіктерде анағұрлым өсіп, 142 О.Т. көрсетті.

Титрлердің геометриялық орташа мәні 35 күндіктерде 21 күндіктерге қарағанда 10,92 есе жоғары болды және алынған оң титрдің жоғары үлесі *M. synoviae*-дің аурудың этиологиясымен қатар оның горизонталды жолмен де берілетінін көрсетеді.

Сероконверсияның бұл эволюциясы микоплазмалардың агрессиялық сипатын және олардың патологиялық процесте белсенді қатысу жағдайын бағамдайды. Үй құстарының серологиялық мониторингі инфекцияны бақылау үшін қажет кезде талап етілетін ем-домдық шаралар арқылы ақылға қонымды іс шара болып саналады.

**Түйін сөздер:** *Mycoplasma synoviae*, бройлер балапандар, ELISA.

И. Ианку<sup>1</sup>, Н. Катана<sup>1</sup>, В. Попа<sup>2</sup>, Ж. Деги<sup>1</sup>, К. Паскю<sup>1</sup>, В. Херман<sup>1</sup>, Г. Ильгекбаева<sup>3</sup>

<sup>1</sup>”Король Майкл I Румынии”Банатский университет сельскохозяйственных наук и  
Ветеринарной медицины, Тимишоара, Румыния;

<sup>2</sup>Институт Пастера, Бухарест, Румыния;

<sup>3</sup>Казахский национальный аграрный университет, Алматы, Казахстан

## ОБНАРУЖЕНИЕ АНТИТЕЛ ПРОТИВ MYCOPLASMA SYNOVIAE В СЫВОРОТКЕ КРОВИ У БРОЙЛЕРНЫХ ЦЫПЛЯТ В ИФА

**Аннотация.** Серологическое исследование проводилось для подтверждения диагноза на инфекционный синовит птиц. По эпизоотологическим и анатомо-клиническим данным было подозрение в серии цыплят-бройлеров с изучаемой фермы.

Результаты эпизоотологического обследования были выражены в том, что совокупная смертность, зафиксированная у цыплят-бройлеров, составляла около 25%, вплоть до убойного возраста.

Анатомо-клинические обследования показали, что через 4 недели у некоторых кур разорвалось икроножное сухожилие с последующей невозможностью смещения и разгибания пораженной конечности. Как правило, это изменение было односторонним.

В зонах артрита, на ранней стадии экссудат сустава в возрасте серо-желтого цвета, еще во время хронической эволюции, выпот был каудальным и содержал синовиальную ткань сухожилия.

После интерпретации реакций и обработки результатов в соответствии с программным обеспечением для интерпретации FlockChek® Avian MS Antibody Test Kit были определены для каждого повторного анализа: группа титров, минимальный титр, максимальный титр и среднее геометрическое (GM). Титры были выражены в оптических плотностях (O.D.).

При первом исследовании, в возрасте 21 дня, было выявлено 5 групп титров (0-4), минимальный титр был 0 O.D. и максимальный титр был 3000 O.D. Во втором – через 35 дней, были выявлены 4 титрационные группы (0-3), минимальный титр был 15 O.D. и максимальный титр был 2293 O.D.

В возрасте 21 дня (В I), 22,91% исследованных сывороток были положительными, а в возрасте 35 дней (В II), 26,66% были положительными, вплоть до возраста убоя (42 дня).

Результаты серологического исследования показали, что в возрасте 21 дня среднее геометрическое значение титров имело значение 13 O.D. по сравнению со средними геометрическими титрами в возрасте 35 дней, когда было зарегистрировано значительное увеличение, составившее 142 O.D.

Геометрическое среднее значение титров в 10,92 раза выше в возрасте 35 дней, чем в возрасте 21 дня, и более высокая доля полученных положительных титров показывают как влияние *M. synoviae* на этиологию заболевания, так и ее инфекционность при горизонтальной передаче.

Эта эволюция сероконверсии предполагает агрессивный характер микоплазм и их активное участие в патологическом процессе. Серологический мониторинг домашней птицы является правильной мерой для контроля инфекций с помощью так называемых профилактических процедур, требуемых при необходимости.

**Ключевые слова:** *Mycoplasma synoviae*, цыплята-бройлеры, ИФА.

### Information about authors:

Iancu I., Doctor of Veterinary Science, Lecturer, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", Timisoara, Romania; ionicavet@gmail.com; <https://orcid.org/0000-0001-8913-4942>

Cătană N., Doctor of Veterinary Sciences, Professor, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", Timisoara, Romania; epirovet.tm@yahoo.com; <https://orcid.org/0000-0002-4242-2418>

Popa V., Doctor of Veterinary Science, Senior researcher, Pasteur Institute Bucuresti, Romania; virgilia.popa@gmail.com; <https://orcid.org/0000-0003-0795-2410>

Dege J., Doctor of Veterinary Science, Lecturer, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", Timisoara, Romania; janos.dege@gmail.com; <https://orcid.org/0000-0002-5859-5057>

Pascu C., Doctor of Veterinary Science, Associated Professor, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", Timisoara, Romania; corina.pascu.ro@yahoo.co.uk, <https://orcid.org/0000-0002-1013-0437>

Herman V., Doctor of Veterinary Science, Professor, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", Timisoara, Romania; viorel.herman@fmvt.ro; <https://orcid.org/0000-0001-7118-6488>

Ilgekbayeva G., Doctor of Veterinary Science, Professor, Kazakh National Agrarian University, Almaty, Kazakhstan; [gulnaz66@mail.ru](mailto:gulnaz66@mail.ru); <https://orcid.org/0000-0001-7140-2961>

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