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# Avian mycoplasmosis monitoring in the Russian Federation in 2019

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## SUMMARY

Under the conditions of intensive poultry farming and high concentration of poultry in a limited area mycoplasmoses cause significant economic damage to commercial poultry farming. Of greatest interest are respiratory mycoplasmosis and infectious synovitis, the causative agents of which are *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, respectively. Considering that both diseases are included in the OIE list of notifiable diseases, it is necessary to perform constant control for their spread. The paper presents an analysis of serological test results for antibodies to mycoplasmosis agents in 2019. Six respiratory mycoplasmosis positive samples – and 129 infectious synovitis positive samples were detected when testing 2,401 chicken serum samples by the enzyme-linked immunosorbent assay (ELISA). The results of monitoring tests of samples received from 31 poultry farms (nine RF Subjects) indicate a decrease in the number of *Mycoplasma gallisepticum*-infected stocks and stable *Mycoplasma synoviae* situation. The respiratory mycoplasmosis epidemic situation on indoor poultry farms might have improved due to obtaining the poultry for commercial parent stocks from mycoplasmosis-free sources, better sanitary and hygienic conditions, elimination of the disease provoking factors, and the use of the disease-specific means of prevention in the parent broiler stocks. The infectious synovitis situation remains tense despite the fact that the number of *Mycoplasma synoviae*-infected farms decreased in comparison with the monitoring results for 2015–2018. Most seropositive stocks were detected on the layer farms (50.0% – in 2019). It seems appropriate to increase the amount of tests to be performed and the number of farms covered by testing in 2020 to more fully present the disease spread situation in commercial poultry farming in Russia.

**Key words:** serological monitoring, respiratory mycoplasmosis (*Mycoplasma gallisepticum*), infectious synovitis (*Mycoplasma synoviae*), enzyme-linked immunosorbent assay, specific antibodies.

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# Мониторинг микоплазмозов птиц в Российской Федерации в 2019 году

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## РЕЗЮМЕ

Микоплазмозы в условиях интенсивного ведения птицеводства и высокой концентрации поголовья птицы на ограниченной территории наносят промышленному птицеводству значительный экономический ущерб. Наибольший интерес представляют респираторный микоплазмоз и инфекционный синовит птиц, возбудителями которых являются *Mycoplasma gallisepticum* и *Mycoplasma synoviae* соответственно. Поскольку оба заболевания включены в список notiфицируемых болезней Всемирной организации здравоохранения животных (МЭБ), необходим постоянный контроль за их распространением. В статье представлен анализ результатов серологических исследований на предмет выявления антител к возбудителям микоплазмозов птиц за 2019 г. При тестировании 2401 пробы сывороток крови кур методом иммуноферментного анализа было выявлено 6 проб, поло-

жительных на респираторный микоплазмоз птиц, и 129 проб, положительных на инфекционный синовит. Результаты мониторинговых исследований проб, поступивших из 31 птицеводческого хозяйства 9 субъектов Российской Федерации, свидетельствуют о снижении количества инфицированных *Mycoplasma gallisepticum* стад и стабильной ситуации по *Mycoplasma synoviae*. Улучшение эпизоотической ситуации по респираторному микоплазмозу на птицефабриках закрытого типа может быть связано с комплектованием родительских промышленных стад из благополучных по данному заболеванию источников, изменением санитарно-гигиенических условий, ликвидацией факторов, провоцирующих заболевание, а также с применением средств специфической профилактики в родительских стадах бройлерного производства. Ситуация по распространению инфекционного синовита остается напряженной, несмотря на уменьшение количества хозяйств, инфицированных *Mycoplasma synoviae*, по сравнению с результатами мониторинга 2015–2018 гг. Наибольшее количество серопозитивных стад выявлено в хозяйствах яичного направления продуктивности, их удельный вес в 2019 г. составил 50,0%. Для более полного отражения ситуации по распространению данных инфекций в промышленном птицеводстве России представляется целесообразным в 2020 г. увеличение объема исследований и количества обследованных хозяйств.

**Ключевые слова:** серологический мониторинг, респираторный микоплазмоз (*Mycoplasma gallisepticum*), инфекционный синовит (*Mycoplasma synoviae*), иммуноферментный анализ, специфические антитела.

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## INTRODUCTION

Currently, a significant and urgent problem in poultry farming is infectious diseases of mycoplasma etiology. *Mycoplasma gallisepticum*, which causes respiratory mycoplasmosis and *Mycoplasma synoviae*, which causes infectious synovitis of birds, are of most interest for veterinary medicine [1–3].

Clinically, diseases are manifested by respiratory disorders (shortness of breath, cough, sinusitis, wheezing), conjunctivitis, tendonitis, loss of appetite, weight loss and egg production decrease (Fig. 1–4) [4, 5]. Mycoplasmosis is usually complicated by a secondary viral and bacterial infection. The most common causative agents of such infections are viruses of infectious bronchitis (IBV), infectious

laryngotracheitis (ILT), avian pneumovirus (APV), *Escherichia coli*, *Ornithobacterium rhinotracheale* (ORT), etc. [2, 6].

In countries with developed industrial poultry farming, mycoplasmosis in poultry causes significant economic damage due to emergency slaughter, decreased egg yield (up to 30%) and meat (up to 16%) productivity, as well as culling young and adult birds [7–9].

Due to the high potential for wide mycoplasmosis spread with breeding material (hatching eggs and day-old chickens) during export-import operations, the diseases are classified as economically significant [10, 11].

Most methods for determining the level of antibodies to *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in chicken serum are based on enzyme-linked immunosorbent assay (ELISA). The advantages of the method are its high sensitivity, specificity, the ability to conduct large-scale studies, quick results, small amount of samples tested and the ability to automate almost all stages of the reaction, including result recording and processing.

For many years, the FGBI "ARRIAH" has been performing mycoplasmosis monitoring, because these diseases (respiratory mycoplasmosis, infectious synovitis) are the OIE listed diseases and require constant monitoring of their spread.

The main goal of this research was to analyze the results of serological monitoring of breeding poultry farms in the Russian Federation for avian mycoplasmosis in 2019.

## MATERIALS AND METHODS

**Diagnostic kits.** "Single serum dilution ELISA kit for detection of antibodies to *Mycoplasma gallisepticum*" and "Single serum dilution ELISA kit for detection of antibodies to *Mycoplasma synoviae*" (manufacturer – FGBI "ARRIAH").

**Solutions and reagents.** To prepare the solutions, double distilled water with pH of 6.0 and electrical conductivity of 0.5 S/cm was used. For ELISA we used: buffer solution (conc.) – for dilution of control and test sera and anti-species conjugate; buffer solution (conc.) for inter-stage



Fig. 1. Eggshell apex abnormalities associated with infectious synovitis

Рис. 1. Синдром стекловидной вершины яйца при инфекционном синовите

washes to remove unbound components; anti-chicken IgG immunoperoxidase conjugate; substrate – ABTS solution (2,2-azino-di(3-ethylbenzothiazoline-6-sulfonic acid); stop solution – 5% sodium dodecyl sulfate solution (solution for stopping the reaction).

**Equipment:** Freezer Indesit DF 4180 W (Russia); Bosch refrigerator (Germany); Indesit 4180 W refrigerator (Russia); drying cabinet LP-309 (Russia); air thermostat TV-20 PZ-“K” (Russia); basic pH-meter PB-11 “Sartorius” (Germany); mechanical dozers of variable volume “Biohit” (Finland), “Socorex” (Switzerland); automatic reader “ELx800” (USA); VCT computer (Russia); psychrometric hygrometer “VIT-1” (Russia); combined device “Testo 608-H1” (China); digital thermometer (logger) “Testo-174T” (Germany); stopwatch SDSpr-1-2-000 (Russia).

**Materials for testing:** Blood serum of chickens of different age groups received from poultry farms of the Russian Federation. Along with the sera from breeding and parent stocks, blood serum from commercial poultry was studied to objectively assess the mycoplasmosis situation.

**Statistical data analysis.** Statistical data processing was performed using the computer program “Statistica for Windows” (USA, Release 4.3; Inc., 1993). Result recording, data processing, storage and analysis were carried out using the SINKO-IFA computer program developed at the FGBI “ARRIAH”.

## RESULTS AND DISCUSSION

When monitoring mycoplasma infections of poultry, the main task was to determine diagnostically significant titers of antibodies to the causative agents of respiratory mycoplasmosis and infectious synovitis, while the parent and grandparent chicken stocks (breeding stock) were the key objects of monitoring.

In 2019, as part of monitoring studies, 2,041 ELISA tests were performed.

Serum samples came from 31 poultry farms from 9 Subjects of the Russian Federation. According to the results of laboratory studies, specific antibodies to avian mycoplasmosis were detected: 6 *Mycoplasma gallisepticum* positive samples (0.58%) and 129 *Mycoplasma synoviae* positive samples (12.64%).

### Determination of serological status of farms for respiratory mycoplasmosis

Blood serum samples to be tested for respiratory mycoplasmosis were obtained from 12 layer poultry farms. Samples from two poultry farms were seropositive, which is 16.7% of the total. As for broiler production, test material came from 19 poultry farms. Samples from one poultry establishment were seropositive, which is 5.26% of the total.

The distribution of titers of antibodies to *Mycoplasma gallisepticum* in the tested samples depending on the age of the poultry is shown in Table 1.

An inconsiderable presence of antibodies to *Mycoplasma gallisepticum* in layer poultry was observed when testing sera collected from poultry over 300 days of age (3.3%), which indicates the circulation of the disease causative agent in the stock.

A low level of antibodies to the respiratory mycoplasmosis agent in broiler poultry of less than 40 days of age indicates the “purity” of parental stocks. Starting from day 130 of age, the number of seropositive poultry increased inconsiderably. It should be noted that the improvement in the respiratory mycoplasmosis epidemic

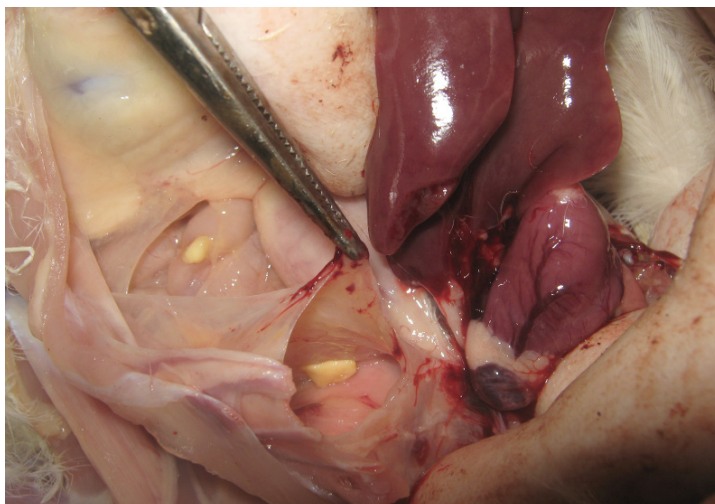


Fig. 2. *Airsacculitis – pathognomonic characteristic of respiratory mycoplasmosis*

Рис. 2. Аэросаккулит – патогномоничный признак респираторного микоплазмоза

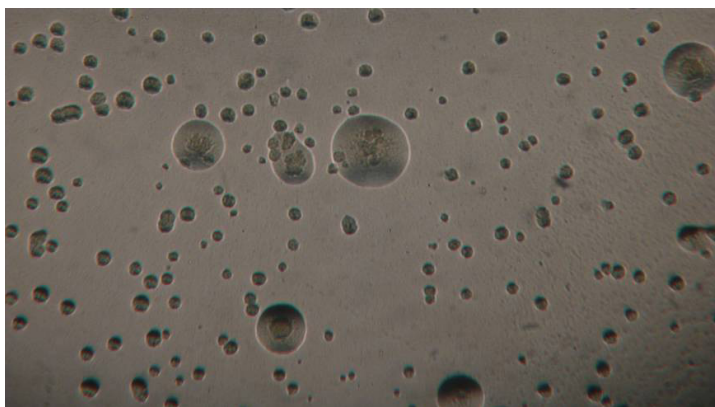


Fig. 3. *Mycoplasma gallisepticum colonies in dense medium*

Рис. 3. Колонии *Mycoplasma gallisepticum* на плотной среде



Fig. 4. *Catarrhal and fibrous sinusitis associated with respiratory mycoplasmosis*

Рис. 4. Катарально-фибринозный синусит при респираторном микоплазмозе



**Table 1**  
Level of antibodies to *Mycoplasma gallisepticum* distributed by age groups on layer and broiler poultry farms in the Russian Federation

Таблица 1  
Уровень антител к *Mycoplasma gallisepticum* по возрастным группам на птицефабриках РФ яичного и бройлерного направления

Age groups, days	Layer farms		Broiler farms	
	Number of positive/ tested samples	ELISA titer	Number of positive/ tested samples	ELISA titer
11–40	n/t	n/t	2/25 (8%)	1,305 ± 14
131–200	0/10 (0%)	0	3/25 (12%)	1,135 ± 20
201–300	0/10 (0%)	0	n/t	n/t
Older than 300 days of age	1/30 (3.3%)	2,370 ± 25	n/t	n/t

n/t – not tested (не исследовали).

**Table 2**  
Level of antibodies to *Mycoplasma synoviae* distributed by age groups on layer and broiler poultry farms in the Russian Federation

Таблица 2  
Уровень антител к *Mycoplasma synoviae* по возрастным группам на птицефабриках РФ яичного и бройлерного направления

Age groups, days	Layer farms		Broiler farms	
	Number of positive/ tested samples	ELISA titer	Number of positive/ tested samples	ELISA titer
1–10	n/t	n/t	4/25 (16%)	1,289 ± 109
11–40	n/t	n/t	11/50 (22%)	1,822 ± 306
41–60	8/25 (32%)	2,416 ± 102	n/t	n/t
61–130	n/t	n/t	n/t	n/t
131–200	11/55 (20%)	1,288 ± 198	n/t	n/t
201–300	5/10 (50%)	1,796 ± 240	n/t	n/t
Older than 300 days of age	90/225 (40%)	2,381 ± 306	n/t	n/t

n/t – not tested (не исследовали).

situation on indoor poultry farms can be associated with obtaining poultry for commercial parent stocks from mycoplasmosis-free sources.

Decrease in the number of positive poultry and the level of antibodies with increasing age indicates improvement in the sanitary and hygienic conditions at the sites and elimination of the disease provoking factors. Thus, according to the results of recent monitoring (2012–2017), the number of adult (more than 300 days of age) poultry with positive reaction was significantly higher and reached 40%.

#### **Determination of serological status of farms for infectious synovitis**

Blood serum samples to be tested for infectious synovitis were submitted from 12 layer poultry farms. Samples from six poultry farms were seropositive, which is 50% of the total. Seropositive samples were also detected on one of 17 broiler farms, which is 5.9% of the total.

The distribution of titers of *Mycoplasma synoviae* antibodies in the tested samples depending on the age of the poultry is shown in Table 2.

The presence of specific antibodies to *Mycoplasma synoviae* in layer poultry at the age of 41–60 days (32%) is indicative of infectious synovitis circulation in the stock, which was probably transmitted transovarially from the chicken parent stock. An increase in antibody titers after transferring replacement stock to the commercial or parent stocks (130 days of age) also indicates the exposure of the poultry to the field infection agent. Given the retrospective analysis data, it should be emphasized that the infectious synovitis situation, unlike respiratory mycoplasmosis, continues to be tense, although it tends to improve. Therefore, in 2018 the proportion of infected households was 57.1%, in 2017 – 66.7%, in 2016 – 78.6%, and in 2015 – 90.32%.

The data specified in the Table 2 demonstrate that the situation on infectious synovitis in the commercial broiler farming is similar. It should be noted that the level of specific antibodies to the infectious synovitis agent prior to transferring to the productive stock is diagnostically insignificant, however, an increase in titers is observed with the age of the poultry, which may be associated with the activation of latent infection.

## CONCLUSION

According to the results of the tests performed, a trend toward noticeable decrease in the number of *Mycoplasma gallisepticum* infected stocks was observed. Seroprevalence of the respiratory mycoplasmosis agent in poultry on layer farms in 2019 was 16.7%, which is 21.3% lower than in 2018. The stabilization of the epidemic situation may be associated with delivering poultry from the disease-free sources, with improvement of sanitary conditions on farms, elimination of stress factors, and use of adequate antibiotic therapy. It is well known that specific prophylaxis carried out in the broiler parent stocks reduces transovarial transmission of the agent, so its use could also improve the epidemic situation. Despite an inconsiderable improvement in the epidemic situation comparing with the monitoring results of 2015–2018 (as there is decrease in the number of *Mycoplasma synoviae* infected farms) and given the similarity of monitoring performed on farms each year, the infectious synovitis situation remains tense. The largest number of seropositive stocks was found on the layer farms (50.0%) in 2019. However, poultry farms maintain high production rates due to the asymptomatic course of infection, which is mainly associated with the prevention of technological and feed stress, which delay clinical disease manifestations.

Considering the fact that respiratory mycoplasmosis and infectious synovitis are included in the OIE list of notifiable diseases due to their cross-boundary distribution with the breeding material, it seems appropriate to increase the amount of tests and the number of farms covered by testing in 2020 to more fully reflect the disease spread situation in commercial poultry farming in Russia.

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