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Evaluation of the effectiveness of blood-drop agglutination test for chicken tuberculosis diagnosis

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SUMMARY

Due to the increased role of opportunistic infections, mycobacterioses, parasitocenoses, etc. the detectability of nonspecific reactions to PPD-tuberculin has sharply increased, which makes it difficult to make a diagnosis and brings laboratory test methods to the fore. The aim of the study was to determine practical significance of blood-drop agglutination test in comparison with allergy test, and frequency of avian tuberculosis lesions on internal organs. For comparative assessment of these techniques 4,086 chickens were tested, including 2,000 young chicks aged 6–9 months and 2,086 adult poultry. In order to compare the results of allergy and serological tests, necropsy was performed for reacting chickens, identified using blood-drop agglutination test and demonstrating positive results using both methods. Low effectiveness of the allergy test in comparison with the serological test was established. The blood-drop agglutination test made it possible to additionally identify 311 adult chickens seropositive for tuberculosis in poultry farms. The effectiveness of this serological method in young birds and poor matching of results in comparison with an allergy test have been shown. The necropsy findings confirmed the practical significance of the serological test; generalized tuberculosis process was noted in all birds positively reacting in blood-drop agglutination test. The dependence of internal organ lesions on poultry-keeping conditions was determined in tuberculosis-affected farms in the autumn and spring periods. Internal organ lesions were found in 835 birds out of 1,072 tested poultry. In the autumn period the intestines were affected in most cases (57.2%), lung lesions were found in the least cases (8.2%), and in the spring period tuberculosis lesions were more often detected in the lungs (43.8%), less often in the intestines (35.5%). In the winter period, the morbidity predominantly occurs due to dust infection, and in summer, birds become infected via alimentary route, which explains the results obtained. The identification of a significantly larger number of diseased chickens, both in advanced form and at an early stage, makes it possible to recommend a blood-drop agglutination test for the diagnosis of tuberculosis. The involvement of internal organs directly depends on the poultry keeping system and should be taken into account when veterinary and sanitary measures are performed.

Keywords: tuberculosis, chickens, blood-drop agglutination test, necropsy, poultry farming, diagnosis, PPD-tuberculin, antigen, blood

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Оценка эффективности кровяно-капельной реакции агглютинации при диагностике туберкулеза кур

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РЕЗЮМЕ

В связи с возросшей ролью оппортунистических инфекций, микобактериозов, паразитоценозов и др. резко увеличилась выявляемость неспецифических реакций на ППД-туберкулин, что затрудняет постановку диагноза и выводит на первый план лабораторные методы исследования. Целью настоящей работы явилось определение практической значимости кровяно-капельной реакции агглютинации в сравнении с аллергической пробой и частоты поражения внутренних органов при туберкулезе птиц. Для проведения сравнительной оценки данных методов было исследовано 4086 кур, из них 2000 гол. составлял молодняк 6–9-месячного возраста и 2086 гол. – взрослая птица. В целях сопоставления результатов аллергических и серологических исследований проводили патолого-анатомическое вскрытие кур из числа реагирующих на аллерген, выявленных с помощью кровяно-капельной реакции агглютинации и положительно реагирующих по обоим методам. Установлена низкая эффективность аллергической пробы в сравнении с серологической реакцией. Кровяно-капельная реакция агглютинации позволила дополнительно выявить в птицеводческих хозяйствах 311 серопозитивных на туберкулез взрослых кур. Показана эффективность данного серологического метода и на молодняке птиц с низким совпадением результатов в сравнении с аллергической пробой. Результаты патолого-анатомического вскрытия подтвердили практическую значимость серологической реакции, во всех случаях у положительно реагирующих в кровяно-капельной реакции агглютинации особей отмечена генерализация туберкулезного процесса. Зависимость пораженности внутренних органов от условий содержания птицы определяли в неблагополучных по туберкулезу хозяйствах в осенний и весенний периоды. Выявили

поражение внутренних органов у 835 гол. из 1072 исследованных особей. В осенний период в большинстве случаев был поражен кишечник (57,2%), в меньшинстве – легкие (8,2%), а в весенний период туберкулезные изменения чаще обнаруживали в легких (43,8%), реже – в кишечнике (35,5%). В зимний период содержания главная причина заболеваемости – пылевая инфекция, в летний же период птицы заражаются алиментарным путем, чем и объясняются полученные результаты. Выявление значительно большего количества больных кур, причем как в запущенной форме, так и на ранней стадии, дает возможность рекомендовать кровяно-капельную реакцию агглютинации для диагностики туберкулеза. Пораженность внутренних органов находится в прямой зависимости от системы содержания птицы и должна учитываться при проведении ветеринарно-санитарных мероприятий.

Ключевые слова: туберкулез, куры, кровяно-капельная реакция агглютинации, патолого-анатомическое исследование, птицеводство, диагностика, ППД-туберкулин, антиген, кровь

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INTRODUCTION

Despite the fact that most of the commitments and goals set within animal tuberculosis control programs have been achieved, tuberculosis in both animals and humans currently remains one of the most complex and significant concerns [1–3].

After the change of socio-economic formation, disruption of established links in the livestock management system and a sharp decline in the industry's potential, it became urgent to develop effective methods for control of infectious diseases, in particular avian tuberculosis [4–6].

According to some authors, with the transfer of poultry farming to commercial basis and reduction in poultry raising time period under optimal veterinary and sanitary conditions on complete feed, the problem of avian tuberculosis has become less notable [7, 8].

At the same time, the emerging increase in the number of poultry in private farms, where uncontrolled and unsystematic movement of both poultry and products is typical, causes certain difficulties in carrying out animal health, managerial and anti-tuberculosis measures. Under these conditions, poultry can become a source of constant circulation of mycobacteria in the environment [9].

According to the literature data, in some farms tuberculosis-affected chickens became the source of infection in cattle. In certain cases, milkers who kept chickens in backyard farms were *Mycobacterium avium* vectors [10–17].

In this regard, timely detection of chickens with tuberculosis is of great practical importance for improving the health of farms affected with this disease. The currently practiced diagnostic methods do not allow detecting all birds with tuberculosis [18–20]. This is evidenced by the fact that after the double tuberculinization and isolation of birds reacting to PPD-tuberculin, chickens with a pronounced form of tuberculosis are subsequently detected in disease-free herds according to necropsy findings [20–24].

The issue of improving and finding new, more effective methods of tuberculosis diagnosis in chickens has been of interest to researchers for a long time. So, for this purpose, scientists began to test serological methods based on immunological tests, for example, the agglutination test. In this regard, a tuberculosis antigen was prepared from avian *Mycobacterium avium* culture [25–32].

Some researchers carried out the hemagglutination test using the blood sera of tuberculosis patients [33–36].

In order to find approaches to the development of more advanced methods of avian tuberculosis diagnosis and taking into account the detectability of chickens with a 50% infection rate among non-reactors to tuberculin, the antigen (strain 9 with pronounced antigenic properties) was prepared for blood-drop agglutination test (BDAT) at the All-Union Institute of Experimental Veterinary Medicine (now the Federal Research Center – All-Russia Research Institute of Experimental Veterinary Medicine named after K. I. Scriabin and Ya. R. Kovalenko of RAS).

In 1955 A. V. Prokhorov et al. conducted BDAT of antigen prepared from *Mycobacterium avium* cultures and compared its results with those of a tuberculin test. At the same time, it was found that a higher number of diseased chickens were detected using the serological method rather than when the allergy test was implemented. Thus, out of 23,355 birds tested using the allergen, 756 (3.2%) birds with tuberculosis were identified. Out of 22,599 non-tuberculin reactors, tuberculosis was diagnosed in 2,079 (8.4%) birds when tested with BDAT [23].

Similar results were obtained by other researchers who consider BDAT to be an effective method of avian tuberculosis diagnosis that can be used for identification of diseased chickens both at the initial disease stage and at the stage of systemic infection [37, 38].

Subsequently, in order to detect a mixed infection (pullorum infection and tuberculosis), a complex antigen was manufactured at the Lithuanian Veterinary Research Institute, which is a mixture of the GNKI pullorum antigen and the tuberculosis antigen of the Lithuanian Veterinary Research Institute [39].

In order to isolate the maximum number of infected birds, many researchers recommend conducting a comprehensive study using two methods: BDAT and tuberculinization [38].

Unfortunately, no data on the scientific significance and practical effectiveness of the blood-drop agglutination test in the diagnosis of chicken tuberculosis is available in the literature. Due to the fact that many aspects of this test's performance remain unstudied, there continues to be a multiplicity of concepts about the justifiability and relevance of this test.

It is important to note that due to the increased role of opportunistic infections, mycobacterioses, parasitocenoses, etc., the detectability of nonspecific PPD-tuberculin reactions has sharply increased, which, of course, complicates the diagnosis and brings laboratory test methods to the fore. In this regard, in order to identify chickens affected with tuberculosis, the necessity of serological test methods is justified, though their role, in our view, is often underestimated.

The aim of the study was to compare the effectiveness of the serological test method (BDAT) with the allergy test, as well as to assess lesions in birds' internal organs in correlation with the chicken keeping system.

MATERIALS AND METHODS

BDAT studies in the diagnosis of avian tuberculosis were carried out in farms of the Republic of Dagestan in the period from 2015 to 2021.

A blood-drop agglutination test using whole blood and antigen was used in combination with intradermal allergy diagnostic testing.

One or two drops of antigen (prepared in the laboratory from a day-old meat-peptone agar culture) were pipetted onto a clean slide. Blood was taken from the axillary vein, transferred to the slides and mixed with the antigen. The reaction was considered positive if the mixture became lighter and flakes were formed due to adhesion of microbes to antibodies, and it was considered specific, if agglutination occurred within one minute.

For comparative assessment of BDAT and allergy test 4,086 chickens were tested, including 2,000 6–9 month-old young birds and 2,086 adult birds.

In order to compare the results of allergic, serological and post-mortem tests, 300 chickens were subjected to diagnostic slaughter: 100 birds reacting to allergen, 100 birds identified with BDAT and 100 birds having positive reactions in both diagnostic tests.

To establish the correlation between internal organ lesions and the poultry keeping system, 1,072 birds were slaughtered: 579 – in the autumn, 493 – in the spring.

Allergy tests were carried out in accordance with the "Guidelines for the use of (PPD) tuberculins in mammals and birds"[1] using avian PPD-tuberculin via intradermal inoculation into the wattle at a dose of 0.1 mL. The test result (the formation of swelling at the injection site) was read after 30–36 hours.

During the simultaneous test, two allergens (dry purified tuberculin and dry purified complex allergen from atypical mycobacteria, CAM) were intradermally inoculated into both sides of the chicken wattles at a dose of 0.1 mL: after that differences in severity of reactions to these allergens were determined[2].

All animal handling procedures were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123).

RESULTS AND DISCUSSION

Comparison of the effectiveness of the BDAT and the allergy test was carried out in farms with the capacity of 2,086 adult poultry considered relatively free from tuberculosis.

The total number of adult poultry in OOO "Buynaksky poultry farm" was 3,678 birds, no PPD-tuberculin reactors were detected there according to the results of scheduled allergy tests over the past 5 years. There were 1,200 animals in the farm SPK "Buglen", positive tuberculin reactors were detected, but the diagnosis was not confirmed later. The diagnosis was not confirmed among adult poultry (768 birds) in the farm "Kazbek" either. No positively reacting birds were found in the flock of 630 birds in the IP "Ruguzh". The test results for adult poultry are shown in Table 1.

It was established that more diseased birds were additionally detected using the BDAT: in OOO "Buynaksky poultry farm" – 3 birds, SPK "Buglen" – 78 birds, KFKh "Kazbek" – 227 birds and IP "Ruguzh" – 3 diseased chickens. The matching of the results of the allergy test and BDAT was noted in 73.90–75.40% of cases.

Testing of young animals of 6–9 months of age was carried out in two farms: on the poultry farms OOO "Karabudakhkentskaya" and at KFKh "Tarki", where over the past 5 years no poultry was subjected to allergy tests. It was possible to identify both seropositive and positively responding to tuberculin birds.

As the results presented in Table 2 show, tuberculosis can be detected with BDAT in birds at an early age. So, 18 birds (1.3%) were detected serologically at the poultry farm OOO «Karabudakhketskaya», and 12 diseased birds (2.0%) were detected in the farm KFKh "Tarki". The matching of BDAT and the allergy diagnostic test results in young animals ranges from 12.5 to 27.8%.

It should be noted that the sensitivity of BDAT at the poultry farm OOO "Karabudakhkentskaya" was higher. Comparing allergy test and serological test results, it can be concluded that a high level of seropositivity indicates the threat of reactivity of the tuberculosis latent form. The high information value of specific antibody detection using BDAT gives grounds to use this test for monitoring in the system of comprehensive infection prevention.

The necropsy results for poultry that reacted to the allergen and were identified using BDAT are shown in Table 3.

During necropsy of positively reacting chickens tested with the BDAT, the tuberculosis systemic process was noted in 13% of the tested birds; lesions of the spleen and intestine were observed in 26% of birds, of liver and intestine – in 14% of tested birds, of liver, intestine and spleen –

[1] Guidelines for the use of (PPD) tuberculins in mammals and birds. Available at: http://www.agrozoo.ru/text/vetprep_html/238.html.

[2] Guidelines for conducting a simultaneous allergy test using tuberculin and a complex allergen from atypical mycobacteria (CAM) in the diagnosis of tuberculosis in animals: approved by the Ministry of Agriculture of the USSR of November 27, 1978. Available at: https://base.garant.ru/70526680.

Table 1
Comparative study of agglutination and allergy test in adult poultry

Name of establishment	Number of tested poultry, birds	Diseased chickens identified with				Matching of BDAT and allergy test results	%
		Allergy test, birds	%	BDAT, birds	%		
OOO "Buynaksky poultry farm"	1,221	–	–	3	0.25	–	–
SPK "Buglen"	400	161	40.25	239	59.75	119	73.90
KFKh "Kazbek"	365	69	18.90	296	81.10	52	75.40
IP "Ruguzh"	100	–	–	3	3	–	–

Table 2
Comparative study of agglutination and allergy test in young poultry

Name of establishment	Number of tested poultry, birds	Diseased chickens identified with				Matching of BDAT and allergy test results	%
		Allergy test, birds	%	BDAT, birds	%		
OOO "Karabudakhkentskaya"	1,400	5	0.36	18	1.30	5	27.80
KFKh "Tarki"	600	16	2.70	12	2.00	2	12.50

in 3% of birds. Tuberculous nodes were detected in the following organs: liver (9%), intestine (7%) and spleen (3%).

During necropsy of chickens identified based only on the results of an intradermal allergic test, systemic tuberculosis was detected in 9% of cases, lesions of the spleen and intestine – in 24% of cases, liver and intestine – in 16% of cases, liver, spleen and intestine – in 1% of cases. As for other organs, lesions were detected in the liver (3%), intestine (4%) and spleen (2%).

Necropsy of chickens with positive reactions detected during diagnostic testing performed by both methods showed the presence of systemic infection in 12%, lesions of the spleen and intestine in 18%, liver and intestine in 15%, liver, spleen and intestine in 2%, liver, intestine and oviduct in 4%, liver, intestine and lymphoid tissues in 1% of the birds subjected to examination. Tuberculous nodes were observed in some organs: liver (6%), intestine (2%) and spleen (1%).

In general, tuberculosis in chickens is accompanied by internal organ lesions typical for this disease. Yellowish-gray or grayish-white tubercles of various shapes and sizes are noted. At the initial stage, some organs are affected with the formation of nodes ranging in size from a poppy seed to a pinhead, clearly visible and separating from healthy tissue.

In advanced cases, there are tuberculous tubercles in several or all internal organs. Sometimes solid knots reaching the size of a hazelnut are formed. Large nodes are often located close to each other, they can merge, forming conglomerates up to 4 cm in size.

Lesions are often observed in several organs, primarily the liver, spleen, and then others, with uniform nodules of the same size.

In general, tuberculosis of chickens is accompanied with patchy lesions in internal organs.

In connection with the above, it seemed reasonable to study correlations between internal organ lesions and

Table 3
Comparison of parameters of internal organ lesions in chickens with tuberculosis

Lesion location	Positive results obtained using		
	BDAT	allergy test	allergy test + BDAT
Systemic form	13	9	12
Spleen and intestine	26	24	18
Liver and intestine	14	16	15
Liver	9	3	6
Intestine	7	4	2
Liver, intestine and spleen	3	1	2
Spleen	3	2	1
Liver, intestine and oviduct	–	–	4
Liver, intestine and lymphoid tissues	–	–	1
There were no macroscopically visible lesions	25	41	39

Table 4
Necropsy findings

Affected internal organs	Infection cases according to the study period			
	autumn		spring	
	number	%	number	%
Liver	83	16	33	10.2
Spleen	27	5.2	18	5.6
Intestine	26	5	2	0.6
Liver and intestine	122	23.4	22	6.8
Systemic form	74	14.6	22	6.8
Liver, spleen and intestine	93	18.5	61	18.9
Intestine and spleen	27	6.4	–	–
Liver, spleen and lungs	27	5.2	32	9.9
Intestine, mesentery and lungs	10	1.9	–	–
Intestine and lungs	8	1.5	–	–
Oviduct	6	1.1	–	–
Spleen and lungs	3	0.5	2	0.6
Liver, spleen and intestine	3	0.5	2	0.6
Muscles	2	0.4	–	–
Liver and lungs	1	0.38	28	14.9
Lungs	2	0.4	14	4.3
Liver, intestine and lungs	–	–	8	2.4
Liver, lungs and oviduct	–	–	12	3.7
Liver and gizzard	–	–	2	0.6
Liver, spleen and oviduct	–	–	10	3
Lungs and kidneys	–	–	2	0.6
Lungs, liver and kidneys	–	–	12	3.7

the poultry keeping system. For that, poultry in tuberculosis-affected farms were tested after winter and summer periods – in spring and autumn. The results are shown in Table 4.

It has been established that lesions of internal organs were detected in 796 birds out of 1,072 birds subjected to diagnostic slaughter at different times. According to the frequency of lesions, the liver, intestine and spleen come first, however many cases of systemic tuberculosis process are also recorded. Along with this, tuberculous lesions are observed in the lungs, oviduct, kidneys, gizzard, cloaca.

As the results of the post-mortem dissection conducted in the autumn period showed, tuberculous nodes were most often found in the intestine (56.2%), less frequently in the lungs (8.9%), and as regards the spring period: in most cases – in the lungs (39.0%) and in the least cases – in the intestine (33.6%). The poultry keeping conditions are likely to be the reason for this. In winter, dust infection seems to prevail, and in summer, birds become infected via alimentary routes.

CONCLUSIONS

1. For avian tuberculosis diagnosis, it is necessary to conduct a comprehensive study with simultaneous performance of a blood-drop agglutination test and a double intradermal allergy test.

2. The blood-drop agglutination test makes it possible to identify a significantly larger number of chickens with tuberculosis both at the systemic infection stage and at an early stage of the disease.

3. The liver, spleen, intestine in birds with tuberculosis are most often affected, and other organs get affected to a lesser extent.

4. The lesions in the intestine and lungs are directly correlated with the poultry keeping conditions. Lesions in the lungs prevail in the winter period, and lesions in the intestine are more common in summer.

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