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Scientific justification of *Mycobacterium avium* survival in natural environment of Republic of Dagestan

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ABSTRACT
Contamination of the environment with the infectious animal disease agents is still a pressing problem for the poultry farms. *Mycobacterium avium* can grow and replicate in organic wastes from the poultry farms for a long time thus contaminating vast adjacent areas and being the source of infection not only for wild and domestic animals but also for humans. The studies were aimed at the examination of the duration of *Mycobacterium avium* survival in the natural environment in two geographical regions of the Republic of Dagestan characterized by different soil and climate. Samples of *Mycobacterium avium*-contaminated feces and soil collected from pastures and farmyards (on the surface and from 5 cm depth) were tested. The experiments demonstrated that pathogenic for chickens mycobacteria survived for up to 30 days in the samples collected in the sub-mountainous areas in summertime, when the air temperature ranged from 15.1 to 30.0 °C, land surface temperature – from 17 to 38 °C, air humidity – from 44 to 94% and average monthly precipitation amounted to 1.5 mm. From September to May, with the air temperature ranging from –10.8 to +25.0 °C, land surface temperature from –14 to +30 °C, air humidity 26–100% and average precipitation 0.39 mm, the bacteria survived for up to 213 days on the soil surface on the pastures and farmyards, and for up to 243 days at the depth of 5 cm and in the feces. In the plain area, in the same time period in the slightly saline soil with high humus content and at air temperature from –11.9 to +27.3 °C, soil temperature from –13 to +45 °C, air humidity from 37 to 100% and average precipitation 20.4 mm, *Mycobacterium avium* survived just like in the sub-mountainous area, i.e. for 213 and 243 days, respectively. The post-mortem lesions in the internal organs of the poultry corresponded to the tuberculosis clinical signs in 86 of 171 birds (50.3%). The study results will allow for the development of the optimal algorithm for animal health and management measures aimed at tuberculosis eradication on the poultry farms.

Keywords: *Mycobacterium avium*, tuberculosis, samples, soil, feces, poultry farm, pastures, stability, infection, contamination, source of infections

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Научное обоснование полиадаптогенности микобактерий птичьего вида в объектах внешней среды в условиях Республики Дагестан

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РЕЗЮМЕ
Для птицеводческих хозяйств актуальной проблемой остается загрязнение окружающей среды возбудителями инфекционных болезней. Микобактерии туберкулеза птичьего вида могут длительное время расти и размножаться в органических отходах птицефабрик, загрязняя огромные примыкающие территории и являясь при этом источником заражения не только диких и домашних животных и птицы, но и человека. Целью работы являлось изучение продолжительности жизнеспособности *Mycobacterium avium* в объектах внешней среды в двух географических зонах Республики Дагестан с разными почвенно-климатическими характеристиками. Исследованию подверглись контаминированные микобактериями птичьего вида пробы (поверхностные и с глубины 5 см) почвы пастбищ, выгульного двора и помета. Эксперименты показали, что в образцах из предгорной зоны, отобранных в летнее время, когда температура воздуха колебалась от 15,1 до 30,0 °С, поверхности почвы – от 17 до 38 °С, влажность воздуха – от 44 до 94% и среднемесячное количество осадков составляло 1,5 мм, жизнеспособность патогенных для кур микобактерий сохранялась до 30 дней. С сентября по май при температуре воздуха от –10,8 до +25,0 °С, почвы от –14 до +30 °С, влажности воздуха от 26 до 100% и среднем количестве осадков 0,39 мм на поверхности почвы пастбищ и выгульного двора бактерии оставались жизнеспособными до 213 дней, на глубине 5 см и в помете – до 243 дней. В равнинной зоне

в этот же временной период в слабозасоленной почве со значительным содержанием гумуса при температуре воздуха от –11,9 до +27,3 °С, почвы от –13 до +45 °С, относительной влажности от 37 до 100% и среднемесячных осадках 20,4 мм микобактерии птичьего вида выживали как в условиях предгорной зоны – в течение 213 и 243 дней соответственно. Патолого-анатомические изменения во внутренних органах птиц соответствовали клиническим признакам туберкулеза у 86 (50,3%) из 171 особи. Полученные результаты позволят разработать оптимальный алгоритм проведения комплекса ветеринарно-санитарных и организационно-хозяйственных мероприятий для оздоровления птицеводческих предприятий от туберкулеза.

Ключевые слова: микобактерии птичьего вида, туберкулез, пробы, почва, помет, птицекомплекс, пастбища, устойчивость, заражение, загрязненность, источник инфекций

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INTRODUCTION

One of the distinctive features of mycobacteria is their stability in the body and various ecosystem objects, which creates great problems for the epizootological control and diagnosis of tuberculosis. Despite the apparent freedom of the Republic of Dagestan from tuberculosis, the pathogen circulation still continues, *inter alia* in the environment, thus maintaining a tendency to the infection spread on the previously free farms [1, 2].

Commercial poultry farming and hereto related short life cycle of commercial chickens, their keeping and feeding factored in their exterior and health parameters, undoubtedly made the problem of avian tuberculosis less significant [3, 4, 5].

At the same time, due to the high stability of *Mycobacterium avium* in the environment (according to the published data, it survives in the soil at the depth of up to 60 cm for more than 3 years), the chicken tuberculosis epizootic process has not been interrupted. Moreover, the disease cases are also detected in the private sector, where uncontrolled and unsystematic movement of poultry and slaughter products is often practiced without appropriate veterinary control. The diseased poultry, being the main source of infection, *inter alia* for wild birds, expands and complements the natural reservoir [6, 7, 8].

When eradicating the disease on the farms, timely removal of the diseased animals from the herd is important, but the problem lies in the fact that weakened and emaciated birds do not react to tuberculin, so they remain undetected for some time, while the risk of infection of healthy birds is proportional to the duration of cohabitation with the diseased ones [9, 10].

In this regard, research on the dynamic tracking of the tuberculosis agent, *inter alia* in the environment, using the proposed up-to-date methods, may be essential in terms of control and prevention of the disease in chickens [11, 12].

According to some published data, tuberculosis-diseased chickens often become a source of infection for cattle, causing sensitization to PPD-tuberculin for mammals [13, 14, 15].

One of the reasons aiding to the spread of this infection is known to be contaminated outer environment, which deserves special attention during epizootological examination [16].

The study of the tuberculosis agent stability in the soil of pastures and poultry yards and in feces as well as the related question of the infection sources and transmission routes is of great importance, since disinfection of large pastures infected with the excretions of the diseased chickens is difficult for economic reasons. In this regard, in our opinion, the correct approach to solving the issue is to rely on their self-decontamination in a natural way [17, 18, 19, 20].

Given these circumstances, the task was set to find out the duration of *Mycobacterium avium* stability in the soil of the pastures, poultry yards and in feces in two climatic zones of the Republic of Dagestan.

MATERIALS AND METHODS

The work was carried out in the sub-mountainous and plain areas of the Republic. Certain areas with different topography and soil composition were isolated both on surface and at the depth of 5 cm. Strips of silk contaminated with *M. avium* were introduced into the soil in these sites, as well as into the feces. The material was contaminated with the second generation culture of the mycobacteria isolated from the chicken died of tuberculosis.

Samples were collected from these sites to determine the survival rate of the mycobacteria. Each sample was individually crushed in 2 mL of sterile saline solution. The resulted mass was filtered through ashless filter paper; 1 mL of the suspension was intramuscularly administered to seronegative healthy chickens of 2 months of age. The infected chickens were kept isolated. Every month they were checked by allergy and clinical tools. At the same time, inoculums and smears were prepared from the emulsion after its appropriate treatment.

The chickens that reacted positively to the allergen were subjected to diagnostic slaughter, and post-mortem examination was performed. The lesions were used for inoculation on the culture media for the production of the initial culture and for preparation of the smears for *M. avium* detection.

During the research, the basic ethical principles of experiments in animals were followed, which are set out

in the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, March 18, 1986). Report of the Bioethics Commission of the Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center No. 7 of 16 November 2023.

In the sub-mountainous area, the experiments were conducted in mildly alkaline black soil. The soil samples contained carbonic and sulfuric acid anions, chlorine, calcium, magnesium, potassium and sodium cations. The amount of humus amounted to 5.69%.

Two experiments were carried out in the sub-mountainous area. The first one (June – August 2019) was carried out under the following meteorological conditions: air temperature ranged from 15.1 to 30.0 °C, soil surface temperature – from 17 to 38 °C, air humidity – from 44 to 94%, average monthly precipitation – 1.5 mm.

To determine the mycobacteria stability, the test samples were collected on days 30 and 61.

The second experiment was carried out from September 2019 to May 2020 with air temperature fluctuations from –10.8 to +25.0 °C, soil temperature from –14 to +30 °C, air humidity from 26 to 100%. During the experiment, the average precipitation amounted to 0.39 mm. The test samples were collected on days 61, 91, 122, 153, 182, 213 and 243.

In the plain area, the experiments were conducted from September 2019 to May 2020 in slightly saline soil with a significant humus content under the following meteorological conditions: air temperature ranged from –11.9 to +27.3 °C, soil temperature – from –13 to +45 °C, relative humidity – from 37 to 100%, average monthly precipitation – 20.4 mm. The samples contained large amounts of phosphorus and chloride salts.

The samples were tested in a similar way to the experiment conducted in the sub-mountainous area.

RESULTS AND DISCUSSION

In the first experiment conducted in the sub-mountainous area, on day 30 of the tuberculosis agent presence in the feces and soil of the pastures and poultry yard, viable and pathogenic for chicken mycobacteria were found both on the surface and at 5 cm depth.

M. avium could not be detected in any of the samples collected on day 61 (Table 1).

Results of the second experiment demonstrated that *M. avium* died on day 213 on the soil surface of the pastures and poultry yard, and on day 243 at 5 cm depth and in the feces (Table 2).

In the plain area, *M. avium* introduced to the soil surface of pastures and poultry yard also died within 213 days, and at 5 cm depth and in feces – within 243 days.

Table 1
Parameters of *M. avium* survival in sub-mountainous environment (experiment one)

Samples	Day	Air temperature, °C	Soil temperature, °C	Air humidity, %	Average monthly precipitation, mm	Presence of mycobacteria
Soil	30	15.1–30	17–38	44–94	1.5	+
	61	15.1–30	17–38	44–94	1.5	–
Feces	30	15.1–30	17–38	44–94	1.5	+
	61	15.1–30	17–38	44–94	1.5	–

Table 2
Parameters of *M. avium* survival in sub-mountainous environment (experiment two)

Samples	Day	Air temperature, °C	Soil temperature, °C	Air humidity, %	Average monthly precipitation, mm	Presence of mycobacteria
Soil	61	–10.8…+25	–14…+30	26–100	0.39	+
	91	–10.8…+25	–14…+30	26–100	0.39	+
	122	–10.8…+25	–14…+30	26–100	0.39	+
	153	–10.8…+25	–14…+30	26–100	0.39	+
	182	–10.8…+25	–14…+30	26–100	0.39	+
	213	–10.8…+25	–14…+30	26–100	0.39	–
	243	–10.8…+25	–14…+30	26–100	0.39	–
Soil at 5 cm depth and feces	61	–10.8…+25	–14…+30	26–100	0.39	+
	91	–10.8…+25	–14…+30	26–100	0.39	+
	122	–10.8…+25	–14…+30	26–100	0.39	+
	153	–10.8…+25	–14…+30	26–100	0.39	+
	182	–10.8…+25	–14…+30	26–100	0.39	+
	213	–10.8…+25	–14…+30	26–100	0.39	+
	243	–10.8…+25	–14…+30	26–100	0.39	–

Table 3
Macroscopic post-mortem lesions of internal organs of poultry

Quantity of poultry	Tuberculosis lesions of								
	liver	spleen	intestines	liver and spleen	liver and intestines	liver, spleen and intestines	intestines and mesentery	Generalized process	Total
171	9	10	6	9	16	13	7	16	86
%	5.3	5.8	3.5	5.3	9.4	7.6	4.1	9.4	50.3

In order to identify post-mortem changes in internal organs and to establish the role of emaciated and weakened poultry in tuberculosis infection of the disease susceptible poultry, diagnostic slaughter of such poultry in the amount of 171 birds was carried out on several poultry farms. During pre-slaughter clinical examination, attention was paid to exhaustion, lameness, low mobility, drooping wings, loss of feather shine. Some chickens demonstrated gastrointestinal disorders, anemia of earrings and wattles, significant mortality was also reported (Table 3).

The post-mortem examination of 171 weakened and emaciated poultry demonstrated tuberculosis granulomas, especially in parenchymal organs, in 86 birds, which amounted to 50.3%.

The results of the conducted studies have shown that in the sub-mountainous and plain areas of the Republic of Dagestan the *Mycobacterium avian* died within 61 days on the surface of pastures, poultry yard and at 5 cm depth and in feces in summer; and within 243 days in the autumn-winter-spring period.

Thus, it can be noted that when eradicating the disease on the tuberculosis infected poultry farms, it is advisable to slaughter poultry in summer. When poultry is slaughtered in June, the pastures infected with the tuberculosis agent are self-decontaminated in the shortest possible time, i.e. in 61 days. This period coincides with the time when the poultry farm is repopulated with young birds born in the current year. Egg incubation in the Republic is started in February, and in March poultry farms receive chickens that reach 6 months of age in August.

In view of the established terms for the pastures' self-decontamination, the replacement young poultry can be transferred to the poultry houses, from where the poultry will be delivered for slaughter in June and the premises will be disinfected. In such case, the pastures where the infected poultry grazed until June will not pose any danger of infection for the young birds born in the current year.

CONCLUSIONS

The following conclusions and practical recommendations were made as a result of the research.

1. Quarantine of the pastures contaminated with *Mycobacterium avian* should be imposed for 2.5 months in summer and for 8.5 months in autumn.

2. Slaughter of poultry on the infected farms for tuberculosis infection eradication should be carried out in June, since in summer the pastures are self-decontaminated from the tuberculosis within a shorter period of time. When slaughter is performed in such a time, there is a possibility of using the pastures by the replacement young birds born in the current year and the chance of the poultry infection with tuberculosis is excluded.

3. When carrying out the disease eradication activities, all weakened and emaciated poultry should be periodically culled and destroyed in a specially designated place, and veterinary and sanitary measures should be subsequently carried out.

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