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Bovine mycoplasmosis occurrence on livestock farms in the Russian Federation for 2015–2018

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SUMMARY

Mycoplasmosis control remains urgent in view of wide spread of bovine mycoplasmoses in the countries with intensive animal farming and trade relations between the Russian Federation and foreign partners including import of pedigree livestock and stud bull semen. Results of testing 1,186 biomaterial samples (blood, sera, nasal swabs, milk, preputial swabs, vaginal swabs, aborted and stillborn fetuses) collected from animals that demonstrated clinical signs of respiratory and reproductive disorders in 34 different regions of the Russian Federation for 2015–2018 are presented in the paper. The samples were tested with real-time polymerase chain reaction (qPCR) for genomes of the following mycoplasmosis agents: *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Mycoplasma dispar*. As a result, *M. bovis* genome was detected in 10.1% of the samples, *M. bovigenitalium* genome was detected in 8.6% of the samples and M. *dispar* genome was detected in 37.15% of the samples. Also, 927 semen samples submitted from Russian and foreign breeding farms were tested with PCR. Test results showed presence of *M. bovis* and *M. bovigenitalium* genomes in semen samples collected from native bull population. Presented data support Russian scientists' conclusions on wide mycoplasmoses occurrence in cattle in the Russian Federation territory and risk of the disease agent introduction through semen import. All of these highlight the need for control of semen products as a source for mycoplasmosis spread as well as insufficiency of single testing of semen for granting the disease-free status to the breeding farm for genetic material marketing.

Key words: *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Mycoplasma dispar*, polymerase chain reaction, spread, cattle, biomaterials, semen.

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Распространение микоплазмозов крупного рогатого скота на животноводческих фермах в Российской Федерации в период с 2015 по 2018 год

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РЕЗЮМЕ

Учитывая широкое распространение микоплазмозов крупного рогатого скота в странах с развитым животноводством и торговые связи Российской Федерации с зарубежными партнерами, в том числе импорт племенного скота и спермы от быков-производителей, проблема контроля микоплазмозов не теряет своей актуальности. В работе представлены результаты исследования 1186 проб биоматериала (кровь, сыворотка крови, назальные смывы, молоко, смывы с препуции и вагинальные смывы, абортированные и мертворожденные плоды), полученных от животных с клиническими признаками респираторной и/или репродуктивной патологии из 34 различных регионов Российской Федерации в период с 2015 по 2018 г. Указанные образцы были исследованы на наличие геномов таких возбудителей микоплазмозов, как *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Mycoplasma dispar*, методом полимеразной цепной реакции в реальном времени. В результате проведенных исследований геном *M. bovis* был обнаружен в 10,1% проб, геном *M. bovigenitalium* выявлен в 8,6% проб, а геном *M. dispar* регистрировали в 37,15% проб. Также с помощью ПЦР-исследования было протестировано 927 образцов семенной жидкости, поступивших из отечественных и иностранных племенных хозяйств. Полученные результаты показали наличие геномов *M. bovis* и *M. bovigenitalium* в образцах спермы от местного поголовья быков. Представленные данные подтверждают выводы отечественных ученых о широком распространении микоплазмозов среди крупного рогатого скота на территории Российской Федерации и угрозе заноса возбудителей заболевания с ввозимой спермой. Все это указывает на необходимость контроля спермопродукции, как источника распространения микоплазмозов, а также на недостаточность однократного исследования семени для присвоения племенному хозяйству статуса благополучия для реализации генетического материала.

Ключевые слова: *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Mycoplasma dispar*, полимеразная цепная реакция, распространение, крупный рогатый скот, биоматериал, сперма.

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INTRODUCTION

Mycoplasma mycoides subsp. *mycoides* SC (Mmm SC), *Mycoplasma bovis* (*M. bovis*), *Mycoplasma bovigenitalium* (*M. bovigenitalium*) and *Mycoplasma dispar* (*M. dispar*) play a significant role in bovine mycoplasmoses development. High disease incidence has a significant impact on animal health situation in animal industry and results in substantial economic losses in meat and dairy industries [1, 2].

M. bovis is the second most common bovine mycoplasmosis agent after Mmm SC; it is one of the major pathogens causing numerous diseases: inflammation of the respiratory tract, arthritis, keratoconjunctivitis, mastitis, etc. [2, 3]. *M. bovis*-associated mastitis in cattle and buffaloes has been already recognized as a serious problem worldwide [4, 5], and the infection caused by the said pathogen is of steadily growing importance due to increase in the said disease outbreaks in the major dairy producing countries [6–8]. Thus, *M. bovis* was detected in animals in the south-east of France [9] and in Czech Republic [10]. According to available reports, *M. bovis* prevalence in the north of Greece was 8.2% [11], in Poland in cattle population – 76.6% [12]. *M. bovis* prevalence in cattle in South America was slightly lower – 6.2% [13].

M. bovis prevalence heterogeneity in different countries can be accounted for various densities of susceptible animals [9]. Sporadic nature of *Mycoplasma*-associated mastitis in France is accounted for small herd sizes as well as effective management practice.

As the agent can be transmitted through infected milk, animal handling, veterinary and zootechnical procedures [14], it is particularly important that the animals without any clinical signs of the disease could be a source of the infection. The risk of mycoplasmosis outbreaks increases when new animals are introduced into the herd [15].

Likelihood of *Mycoplasma* infection in cattle raised under semi-intensive farming systems is 4.6 times higher than in free-ranging animals [16]. This is due to the fact that the risk of the pathogen transmission via direct contact between animals increases when the animals are reared under semi-intensive systems [17].

M. bovis was isolated in 2.2% of tested vaginal samples taken from cows in Egypt whereas *M. bovigenitalium* detection rate was 13.3% [18]. *M. bovigenitalium* was detected using similar tests in Brazil (9.29%) [19] and Japan (7.4%) [20].

M. bovigenitalium-associated genital infections in cows are characterized by granular vaginitis, vulvovaginitis with mucous and purulent vaginal discharges that could result in infertility and occasionally necrotic endometritis [21].

Economic losses due to *M. bovigenitalium* infection are attributed to infertility and poor reproductive performance of animals [21, 22].

Many researchers believe that *M. dispar* is responsible for bovine respiratory diseases that are widespread and characterized by upper respiratory tract mucosa inflammation and lung lesions. Though microorganisms of the said *Mycoplasma* species cause mild pneumonic lesions increased *M. dispar* occurrence confirms their role in bovine respiratory disease pathogenesis. Under unfavorable conditions mycoplasmas by themselves or in combination with other infectious agents can cause serious respiratory diseases resulting in economic losses in large animal farming holdings with high animal density [23, 24].

The study was aimed at analysis of *M. bovis*, *M. bovigenitalium* and *M. dispar* prevalence in different Subjects of the Russian Federation and tests of native and imported stud-bull semen samples for genomes of the above-said mycoplasmas.

MATERIALS AND METHODS

The following samples were used for tests: blood, sera, nasal swabs, milk, preputial swabs, vaginal swabs, aborted and stillborn fetuses. The samples were collected from animals with clinical signs of respiratory and reproductive disorders in 34 different regions of the Russian Federation for 2015–2018. Tests of 115 biomaterial samples were carried out in 2015; 337 biomaterial samples were tested in 2016; 373 biomaterial samples were tested in 2017 and 361 biomaterial samples were tested in 2018.

Additionally, stud bull semen samples (483 semen straws) obtained from breeding holdings located in different regions of the Russian Federation were tested in the FGBI "ARRIAH" (Vladimir).

Furthermore, 444 semen samples collected from stud bulls in different Russian and foreign breeding centres were tested in the Unit for Gene Diagnosis of Infectious Animal Diseases of the FGBI "VGNKI" (Moscow).

Seminal fluid was periodically collected from four stud bulls with impaired reproductive performance to test for *M. bovis* and *M. bovigenitalium* shedding with semen.

Samples were preliminary processed in accordance with the requirements of the Methodical Guidelines 1.3.2569-09 "Operation procedures for the laboratories using nucleic acid amplification techniques for tests of the materials containing Pathogenicity Group I–IV microorganisms". In the FGBI "ARRIAH" the agent DNA was extracted with AllPrep DNA/RNA Mini Kit (Qiagen, Germany); in the FGBI "VGNKI" the agent DNA was extracted with RIBO-prep kit (AmpliSens, Russia) in accordance with the relevant instruction for use.

PCR assays of the biological materials were performed in the FGBI "ARRIAH" with real-time polymerase chain reaction (qPCR) using own commercial kits for *M. bovis*, *M. bovigenitalium* and *M. dispar* detection in accordance with their instructions for use.

Stud bull semen straw samples were tested in the FGBI "VGNKI" with qPCR in accordance with the methods developed earlier [25].

TEST RESULTS

Tests of the biomaterials submitted from different regions of the Russian Federation

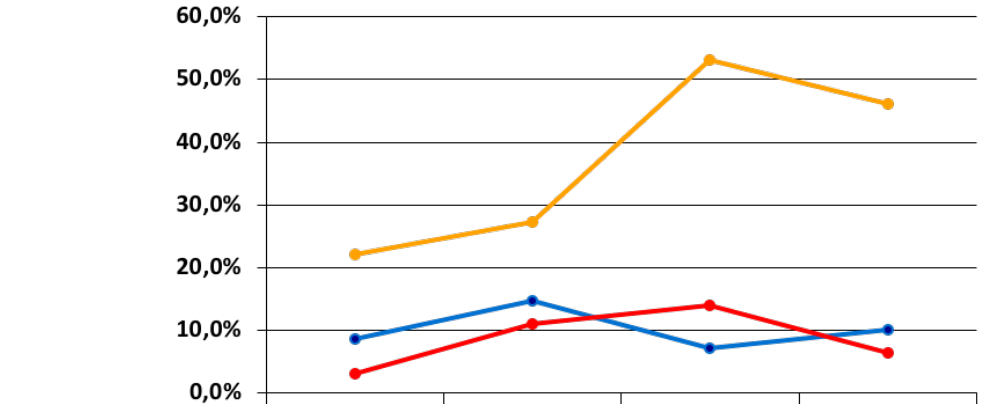
Figure 1 shows results of qPCR tests of 1,186 biomaterial samples collected from cattle in 34 Subjects of the Russian Federation for 2015–2018.

Figure 1 shows that average detection rate of *M. bovis*, *M. bovigenitalium*, *M. dispar* genome was 10.1%, 8.6% and 37.15%, respectively, for the whole test period.

It should be noted that *M. dispar* was detected more often than *M. bovis* and *M. bovigenitalium* based on the analysis of *Mycoplasma*-positive samples. Average percentage of *M. dispar* genome-positive samples out of all samples that had been PCR-positive for *Mycoplasma* for 4 years was 58.75%, whereas for *M. bovis* and *M. bovigenitalium* it was 32.50% and 8.75%, respectively.

Tests of semen samples

A total of 241 semen samples collected from native donor stud bulls and 242 semen samples collected from imported donor stud bulls were tested in the FGBI "ARRIAH" for assessment of seminal fluid quality. Bovine *Mycoplasma*



	2015	2016	2017	2018
M. bovis	8,5%	14,6%	7,2%	10,1%
M. bovigenitalium	3,0%	11,0%	14,0%	6,4%
M. dispar	22,1%	27,2%	53,2%	46,1%

Fig. 1. Results of tests of biological materials for mycoplasma genomes (2015–2018)

Рис. 1. Результаты выявления генома микоплазм в пробах биологического материала в 2015–2018 гг.

Table 1
Results of tests of 483 semen product samples carried out in the FGBI "ARRIAH"

Таблица 1
Результаты исследований 483 образцов спермопродукции, проведенных в ФГБУ «ВНИИЗЖ»

Pathogen	Number of positive semen samples			
	native		imported	
	number of samples	%	number of samples	%
M. bovis	28	11.6	6	2.5
M. bovigenitalium	70	29.0	10	4.1
Total	98	40.6	16	6.6

genomes were detected in 114 semen samples (23.6%) out of 483 semen samples submitted from 13 Subjects of the Russian Federation in 2015–2018.

Data on tests of seminal fluid for *Mycoplasma* contamination are presented in Table 1.

Data given in Table 1 show that in Russian semen samples *M. bovigenitalium* genome was detected most frequently (29%), while *M. bovis* genome was detected only in 11.6% of the samples.

M. bovigenitalium and *M. bovis* genomes were detected in 10 (4.1%) and 6 (2.5%) semen samples, respectively, out of 242 imported semen samples.

Test results for 2015–2018 were analyzed to determine detection rates of *M. bovis* and *M. bovigenitalium* in seminal fluids (Fig. 2).

The analysis revealed that detection rate of *M. bovis* (2.1%) and *M. bovigenitalium* (6.3%) genomes was the lowest in 2015. However, it had increased up to 6 and 15.5%, respectively, by 2018 (Fig. 2). *M. bovis* genome detection rate was maximum in 2016: 9.8% of *M. bovis*-positive samples out of total number of tested samples. The highest number of *M. bovigenitalium* genome-containing samples was detected in 2017: 25% of *M. bovigenitalium*-positive samples out of total number of tested samples.

M. bovis and *M. bovigenitalium* genomes were detected with qPCR in 187 samples (42.1%) out of 444 semen samples obtained from Russian and foreign breeding centres that were tested in the FGBI "VGNKI".

No *M. bovis* genome was detected in semen samples submitted from Russian breeding holdings, whereas *M. bovigenitalium* DNA was detected in 60.7% of tested samples (Table 2).

M. bovigenitalium and *M. bovis* genomes were detected in 22.3 and 3%, respectively, of tested semen straws obtained from breeding holdings located in the UK, USA and Netherlands. Therewith, *M. bovis* DNA was detected only in semen straws submitted from US breeding centres.

Test of stud bulls for Mycoplasma shedding

For tests for *Mycoplasma* shedding with semen, seminal fluid samples were collected from four stud bulls with impaired reproductive performance for 2015–2018. Collected samples were tested for *M. bovis* and *M. bovigenitalium* genomes with qPCR. Test results are given in Table 3.

The results given in Table 3 show that only *M. bovigenitalium* genome was detected in semen collected from the tested bulls. It should be noted that *M. bovigenitalium* genome was detected within the period of 2015–2017 while

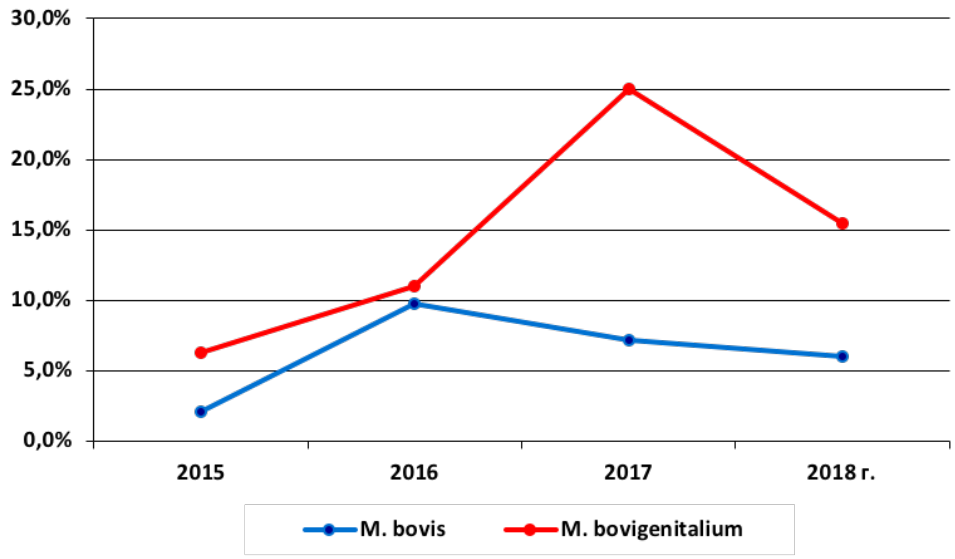


Fig. 2. M. bovis and M. bovigenitalium detection in semen product samples in 2015–2018

Рис. 2. Выявление геномов M. bovis и M. bovigenitalium в пробах спермопродукции в 2015–2018 гг.

Table 2
Results of tests of 444 semen product samples carried out in the FGBI "VGNKI"

Таблица 2
Результаты исследований 444 образцов спермопродукции, проведенных на базе ФГБУ «ВГНКИ»

Pathogen	Number of positive semen samples			
	native		imported	
	number of samples	%	number of samples	%
M. bovigenitalium	128	60.7	52	22.3
M. bovis	0	0	7	3.0
Total	128	60.7	59	25.3

samples collected from the same stud bulls in 2018 were tested negative.

The frequency of *Mycoplasma* genome detection in stud bull semen can be associated with many external and internal factors: introduction of infected animals in the herd, regular antimicrobial treatment, etc. *Mycoplasma* infections have remained understudied so far and require further investigations in the field of their diagnosis and clinical infection in animals.

DISCUSSION

Mycoplasmoses are one of the most common infectious diseases in cattle. About 100,000 new cases of clinical *Mycoplasma* infections are reported annually in cattle in Germany and the USA [26].

M. dispar genome was the most often detected in biomaterials from cattle with clinical signs of respiratory disorders (Fig. 1). It is important to note that while *M. bovigenitalium* is a genital pathogen in some cases its genome has been detected in respiratory organ samples and in nasal swabs. Analysis of detection dynamics shows an upward trend for detection rate of *M. dispar* genome in tested samples (Fig. 1), whereas detection rate of *M. bovis* and *M. bovigenitalium* remains approximately at the same level; this highlights the need for systematic monitoring of the said pathogens.

The fact that mycoplasmas can contaminate stud bull semen is of great concern. Use of uncertified semen can significantly undermine *Mycoplasma* freedom of the whole populations in breeding and/or artificial insemination centres. Results of the test carried out in the FGBI "VGNKI" showed that *M. bovis* genome was detected in 3% of stud bull semen samples from foreign breeding centres and was not detected in stud bull semen samples from Russian breeding holdings. *M. bovigenitalium* DNA was detected in 60.7% of stud bull semen samples from Russian breeding holdings and in 22.3% of stud bull semen samples from foreign breeding centres (Table 2, Fig. 2).

During the tests carried out in the FGBI "ARRIAH", *M. bovis* and *M. bovigenitalium* genomes were detected in 11.6 and 29.0% of semen samples collected from native stud bulls and in 2.5% and 4.1% of imported semen samples, respectively (Table 1).

Notably, there is a difference between results of semen straw tests performed by the FGBI "VGNKI" and FGBI "ARRIAH". This can be accounted for different origin of samples selected for tests. Samples from large-scale animal holdings including those being both production and breeding centres were submitted to the laboratory of the FGBI "ARRIAH". The samples were not separated during the said tests.

Semen purchased directly in the breeding centres was tested in the FGBI "VGNKI". Also, differences in control of stud bull health in Russian breeding centres and foreign breeding centres importing semen straws to the Russian Federation should be considered.

Mycoplasmosis is a factor-associated infectious disease triggered by stress, animal overcrowding, wet conditions, increased air humidity, inappropriate diet, etc.

Antibiotic treatment carried out in the holdings for *Mycoplasma* control contributes to the herd health improvement. However, it is important to understand that this measure alone is not sufficient for the complete recovery of the animals due to long-term *Mycoplasma* persistence in animal body and periodical *Mycoplasma* shedding by the animals. This reveal the need for systematic monitoring of the said infections (Table 3).

Presented data support results of the tests performed by Russian scientists that are indicative of wide mycoplasmoses occurrence in cattle in the Russian Federation and risk of the *Mycoplasma* agent introduction through imported semen [27, 28].

The above-said test results highlight the need for control of semen products (especially imported ones) as a source of *Mycoplasma* spread as well as insufficiency of single testing of semen for granting the disease-free status to the breeding holding for genetic material marketing.

CONCLUSION

Test results are indicative of bovine mycoplasmoses occurrence in the holdings located in different regions of the Russian Federation for 2015–2018. Identification of *M. bovis* and *M. bovigenitalium* genomes in semen from Russian and foreign breeding centres reveals high risk of the further spread of pathogenic mycoplasmas in the absence of systematic surveillance aimed at their spread prevention.

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Table 3
Results of periodic tests of bull semen for *M. bovis* and *M. bovigenitalium* genomes

Таблица 3
Результаты периодических исследований спермы быков на наличие геномов *M. bovis* и *M. bovigenitalium*

No.	Animal No.	Sampling date	Test results	
			M. bovis	*M. bovigenitalium*
1	1	25.07.15	no genome was detected	no genome was detected
2		15.05.16	no genome was detected	no genome was detected
3		05.09.17	no genome was detected	**genome was detected**
4		10.12.17	no genome was detected	no genome was detected
5		27.10.18	no genome was detected	no genome was detected
6	2	14.03.15	no genome was detected	**genome was detected**
7		18.10.15	no genome was detected	**genome was detected**
8		14.03.16	no genome was detected	no genome was detected
9	3	10.04.15	no genome was detected	no genome was detected
10		03.12.15	no genome was detected	no genome was detected
11		21.01.16	no genome was detected	no genome was detected
12		20.10.17	no genome was detected	no genome was detected
13		27.10.18	no genome was detected	no genome was detected
14	4	17.04.15	no genome was detected	**genome was detected**
15		16.07.15	no genome was detected	**genome was detected**
16		02.12.15	no genome was detected	**genome was detected**
17		28.04.16	no genome was detected	no genome was detected
18		07.09.16	no genome was detected	**genome was detected**
19		14.02.17	no genome was detected	no genome was detected
20		13.08.17	no genome was detected	no genome was detected
21		16.12.17	no genome was detected	no genome was detected
22		07.02.18	no genome was detected	no genome was detected
23		26.05.18	no genome was detected	no genome was detected
24		28.09.18	no genome was detected	no genome was detected
25		20.12.18	no genome was detected	no genome was detected

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