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IDENTIFICATION OF ANTIBIOTIC RESISTANCE OF THE CATTLE PATHOGEN *Histophilus somni*

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Abstract

Histophilus somni is a Gram-negative bacterium of the *Pasteurellaceae* family which is a component of the Bovine Respiratory Disease Complex and the pathogen, causing a multisystem disease — histophilosis. For the treatment of diseases caused by *Pasteurellaceae* bacteria, aminoglycosides, sulfonamides, beta-lactams, tetracyclines and macrolides are most often used, therefore the formation of resistance of *H. somni* to antibiotics of these groups can be expected. This work is the first study of antibiotic resistance of *H. somni* isolated from cattle in the Russian Federation. This work aimed at exploration of the antibiotic resistance of circulating *H. somni* strains by phenotypic and genotypic methods and the evaluation of the PCR method applicability for the prediction *H. somni* resistance to antimicrobial agents. We studied 18 cultures of *H. somni*, the causative agent of histophilosis, isolated in 2018-2019 from biological material (parenchymal organs, washes, sperm) of 145 animals of different breed, age, sex and physiological groups using microbiological method. The cultures were studied using the disk diffusion method for sensitivity to 13 antibiotics of aminoglycosides, beta-lactams, tetracyclines and sulfonamides classes. All obtained isolates were tested by PCR for the presence of genetic determinants of antibiotic resistance, most often found in *H. somni*: *tetH* (resistance to tetracyclines), *bla*$_{OXA-2}$ (resistance to penicillins), *aadA25*, *strA*, *strB*, *aphA1* (resistance to aminoglycosides), *sul2* (sulfonamide resistance). Resistance to aminoglycoside group was most prevalent, i.e., resistance to streptomycin was 50 %, and resistance to neomycin exceeded 40 %. Genes *aadA25*, *strA*, *strB* and *aphA1* were found in the resistant samples. A total of 33 % isolates showed resistance to sulfonamides, all this samples were positive for the *sul2* gene in PCR. The sensitivity to penicillins was quite high (~ 75 %), the sensitivity to beta-lactams approached 100 %. The sensitivity to antimicrobials of the tetracycline group was higher than 80 %. However, neither tetracyclines (*tetH*) nor penicillins (*bla*$_{OXA-2}$) resistance genes were identified during the study. Two isolates were multidrug resistant with resistance to aminoglycosides, beta-lactams and tetracyclines. Also, four samples were resistant to antimicrobial agents of two different groups, i.e., two samples were resistant to aminoglycosides and sulfonamides with *strA*, *strB*, *aadA25*, *aphA1*, and *sul2* genes found, and two samples were resistant to aminoglycosides and beta-lactams with only aminoglycoside resistance genes *aadA25* and *strA* identified. With the exception of samples resistant to tetracyclines and beta-lactams, in which the expected genes were not detected, all observed phenotypes of antimicrobial resistance were consistent with the PCR test results. The combination of genotypic and phenotypic methods for determining antibiotic resistance is necessary for understanding of the resistance mechanisms and increases the efficiency of antibiotic resistance monitoring programs.

Keywords: *Histophilus somni*, PCR, antibiotic resistance, histophilosis, cattle

Histophilus somni (the family *Pasteurellaceae*) is a gram-negative bacterium that is often found in cattle and, as a rule, complicates the severity of respiratory viral diseases [1]. *H. somni* can cause a multisystem disease known as histophilosis. The infection of the upper respiratory tract often precedes damage to other organ

systems. *H. somni* is the most common causative agent of respiratory disease and pneumonia in calves aged 1-2 months and it can also cause lung lesions in feedlot animals. The pathogen can persist for a long time in the host, gradually spreading throughout the herd. In the study of respiratory diseases in cattle in the United States and Canada, it was shown that *H. somni* is the second most common (57 %) bacterial pathogen after *Mannheimia haemolytica* (91 %) [2-5].

Antimicrobials are widely used to treat histophilosis and other bacterial infections of cattle, however, the emergence and spread of antibiotic-resistant strains significantly reduces the effectiveness of antimicrobial therapy. Macrolides, tetracyclines, beta-lactams (penicillins and cephalosporins), aminoglycosides, fenicols and sulfonamides [6, 7] are mostly applied against pathogens of the *Pasteurellaceae* family [6, 7], therefore, the appearance of *H. somni* strains resistant to antibiotics of these groups is expectable. Antimicrobial sensitivity of *H. somni* is currently quite high, but in recent years, pronounced resistance has begun to develop to antibiotics of certain groups [5, 8, 9].

The first investigations of antibiotic resistance of *H. somni* were done by the Upjohn Company (USA) in 1988-1992 [10]. The sensitivity of *H. somni i*solates from cattle lungs in the USA and Canada to beta-lactams, tetracyclines, and macrolides exceeded 90 %, to the aminoglycoside spectinomycin was 87.1 %. Only 35.8 % of the samples were sensitive to the sulfamethazine of sulfonamide group [10]. According Welsh et al. [11], in the USA in 1994-2002, the sensitivity of *H. somni* to ampicillin, cephalothin, and tilmicosin remained high (94-100 %), and to tetracycline and spectinomycin decreased to 88-94 and 65 %, respectively. Portis et al. [12] showed that in vitro sensitivity of *H. somni* from the respiratory complex of cattle to beta-lactams remained close to 90 % during 2000-2009. Also, 90-100 % of *H. somni* isolates were sensitive to florfenicol, while the percentage of *H. somni* isolates sensitive to tetracycline decreased over a 10-year period [12]. Studies of *H. somni* resistance in Australia showed 100 % sensitivity of bacteria to ceftiofur of third-generation cephalosporins, florfenicol, and enrofloxacin, while one sample was resistant to macrolides [13].

From 2012 to 2016, monitoring of *H. somni* in Canada revealed resistance to the aminoglycoside neomycin in 93.6 % of isolates [14]. Lamm et al. [15] reported a wide variability in the sensitivity of the *Pasteurellaceae* family members, the *Pasteurella multocida*, *M. haemolytica*, and *H. somni* to the macrolide tilmicosin (88, 42, and 0 %, respectively). The sensitivity of all three species to antibiotics of the fluoroquinolone group was high (90-98 %) and to tetracycline low (40 %) [15].

Studies of *H. somni* resistance to antibiotics are carried out mainly in the USA and Canada [4, 5, 12]. In Europe, the problem of *Pasteurellaceae* resistance is paid less attention. Despite the fact that the countries of the European Union have implemented two antibiotic resistance monitoring programs — the ARBAO-II organized by the EU in 2003-2005 [16] and VetPath supervised by the European Animal Health Study Center (CEESA, Belgium) in 2002-2006 [17], the resistance of *H. somni* has not been practically studied. Within the framework of these programs, the sensitivity of *P. multocida* and *M. haemolytica* to various antibiotics was investigated. For sensitivity of these bacteria to florfenicol, ceftiofur, and the combination of amoxicillin with clavulanic acid, similar results were obtained in both programs, but the data on the sensitivity to tetracycline are very different [16, 17]. Though *H. somni*, *P. multocida*, and *M. haemolytica* belong to the same family, they demonstrate different sensitivity to antimicrobials [7, 10, 15] which does not allow drawing parallels between them with regard to the development of antibiotic resistance. Russian researchers also evaluated the antibiotic resistance of *P. multocida* and *M. haemolytica* isolated from cattle [18], however, the antibiotic

resistance of *H. somni* has not been studied.

Currently, genetic determinants of antibiotic resistance of bacteria causing cattle respiratory diseases have received much attention [19, 20]. For microorganisms of the *Pasteurellaceae* family that are of veterinary importance (*Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, *Histophilus*), the presence of at least 9 genes associated with resistance to tetracyclines, 5 genes with resistance to beta-lactams, 6 genes for macrolides, and 10 for aminoglycosides are reported [21]. D'Amours et al. [22] who sought for various variants of tetracyclines resistance genes in *H. somni* isolates identified only the *tetH* gene but no other genes. In the papers of Canadian and American researchers, there are data on the integrative conjugative element (ICE) which carries several genes at once encoding the resistance of *H. somni* to antibiotics [14, 19, 25]. *H. somni* has the resistance genes *tetH* for tetracyclines, *bla*$_{OXA-2}$ for penicillins, *strA*, *strB*, *aadA25*, *aphA1*, and *aadB* for aminoglycosides, *sul2* for sulfonamides, *erm(42)*, *mrs(E)-mph(E)* for macrolides, *floR* for florfenicol, and *dfrA14* for trimethoprim [19, 21].

This work is the first to characterize resistance to antimicrobials of *H. somni* strains isolated from cattle in the Russian Federation. Circulation of resistant *H. somni* strains within the Russian livestock farms has been established for the first time.

Our goals were to study the antibiotic resistance of circulating *Histophilus somni* strains using phenotypic and genotypic techniques and to assess if the PCR analysis allows predicting the resistance of *H. somni* to several groups of antimicrobial agents. Assessment of antibiotic resistance was based on molecular identification of genes *blaOXA-2*, *sul2*, *strA*, *strB*, *aadA25*, *aphA1*, and *tetH*.

Materials and methods. *H. somni* cultures were isolated in 2018-2019 from biological material (parenchymal organs, washes, sperm) of cattle of different breeds, age, sex, and physiological groups. The samples were used for culturing on the nutrient medium CM0898B (Oxoid™, Thermo Fisher Scientific, USA) with the supplement FD117 (HiMedia Laboratories Pvt, Ltd., India) and on chocolate agar. Bacteria were grown in 10 % CO_2 at 37 °C for 48 hours. Susceptibility of *H. somni* isolates to streptomycin (10 μg), neomycin 30 (μg), ampicillin (10 μg), amoxicillin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), tetracycline (30 μg), doxycycline (30 μg) (HiMedia Laboratories Pvt., Ltd., India) were tested by disk diffusion method, susceptibility categories (susceptible, resistant, or intermediate) was determined by growth inhibition zones according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [23] with modifications.

Bacterial suspensions were prepared in brain-heart broth from 48-hour agar cultures. The turbidity of the suspensions was adjusted to 0.5 McFarland standard (1.5×10^8 CFU/cm^3). For antimicrobial susceptibility testing by disk diffusion, the isolates were incubated for 24 hours at 37 °C in 8-10 % CO_2. The diameter of the inhibition zone was measured with a caliper and expressed in millimeters. All measurements were carried out in 3 replicates.

To identify genetic determinants of antibiotic resistance in *H. somni*, DNA was extracted from a 100 mm^3 suspension using a DNA-sorb-B reagent kit (Central Research Institute of Epidemiology, Russia). *H. somni* isolates were tested for the presence of seven antibiotic resistance genes, *tetH*, *bla*$_{OXA-2}$, *aadA25*, *strA*, *strB*, *aphA1*, and *sul2* by conventional and Real-time PCR [24]. Conventional PCR was carried out according to the following program: 5 min at 95 °C; 10 s at 95 °C, 20 s at 55-64 °C (depending on the primers used), 10 s at 72 °C (40 cycles); 3 min at 72 °C (a Tertsik thermocycler, DNA-Tekhnologiya, Russia). Reaction mixtures for PCR of total 25 mm^3 volume contained 10 mm^3 template DNA, 6 pmol of

each specific primer, dNTPs, and PCR-mixture-2-blue (AmpliSens, Russia). Detection was carried out by electrophoresis in 1.5 % agarose gel. Positive samples were additionally confirmed by Sanger sequencing.

To identify genes *aadA25*, *strA*, *strB*, and *sul2*, TaqMan fluorescent probes were designed and Real-time PCR conditions were optimized. PCR on a Rotor-Gene 6000 (Corbett Research Pty, Ltd., Australia) and a Rotor-Gene Q (Qiagen, Germany) was performed as follows: 15 min at 95 °C; 10 s at 95 °C, 20 s at 63 °C, 10 s at 72 °C (5 cycles without fluorescent signal detection); 10 s at 95 °C, 20 s at 60 °C, 10 s at 72 °C (35 cycles with fluorescent signal detection). Amplification of the *strA* and *strB* genes was carried out simultaneously in a single multiplex real-time PCR assay.

The Real-time PCR was performed in a 25 mm^3 volume reaction mixtures containing 10 mm^3 template DNA, 10 mm^3 of PCR mixture-1 (6 pmol of each specific primer and 3 pmol of each probe, dNTPs, deionized water), 0.5 mm^3 of Taq-F polymerase, and 5 mm^3 of PCR-buffer-Flu (AmpliSens, Russia). The efficiency of DNA extraction was assessed by amplification of internal exogenous controls (IECs). Recombinant plasmids with target inserts (PCR products) into the vector plasmid pAL2-TA (Evrogen, Russia) were used as positive PCR controls.

PCR products were sequenced using Big Dye® Terminator v1.1. Cycle Sequencing Kit (Applied Biosystem, USA), a GeneAmp PCR System 2720 amplifier (Applied Biosystem, USA) and an ABI PRISM 3130 Genetic Analyzer automatic sequencer (Applied Biosystem, USA).

Results. From 145 animals 18 *H. somni* cultures were isolated and identified. Since *H. somni* is a slow-growing organism demanding on the conditions of cultivation, we managed to grow only 12 samples to the counts enough to be evaluated for antibiotic susceptibility.

The investigated isolates were mostly resistant to aminoglycosides. The resistance to streptomycin was 50 %, and resistance to neomycin exceeded 40 % (Table 1). Four samples were additionally tested for susceptibility to kanamycin, gentamicin and spectinomycin. Kanamycin resistance was found in one sample. All 4 samples were susceptible to gentamicin and spectinomycin. In the works of American researchers, only one antibiotic of the aminoglycoside group, the spectinomycin, was included in the panel of antibiotics in a susceptibility testing [10-12, 15]. The susceptibility of *H. somni* isolates to spectinomycin decreased from 86 % in 1988-1992 to 65-86 % in 1994-2002 [10, 11]. Bhatt et al. [14] also showed a high susceptibility of *H. somni* to spectinomycin (87 %), however, Canadian isolates, 22 % of collected during 1980-1990 and 93.6 % collected in 2012-2016, appeared to be resistant to another aminoglycoside antibiotic neomycin, indicating an increase in resistance to this drug.

1. Phenotypic resistance of *Histophilus somni* isolates in antimicrobial susceptibility testing by disk diffusion method

Group of antibiotics	Drug	Number (%)		
		resistant isolates	intermediate isolates	susceptible isolates
Aminoglycosides	Streptomycin	6 (50 %)	–	6 (50 %)
	Neomycin	5 (42 %)	2 (16 %)	5 (42 %)
Beta-lactams	Ampicillin	3 (25 %)	–	9 (75 %)
	Amoxicillin	3 (25 %)	–	9 (75 %)
	Cefotaxime	1 (8 %)	–	11 (92 %)
	Ceftazidime	–	–	12 (100 %)
	Ceftriaxone	–	–	12 (100 %)
Tetracyclines	Tetracycline	–	–	12 (100 %)
	Doxycycline	2 (17 %)	–	10 (83 %)

N o t e. Dashes indicate the absence of isolates resistant to the antibiotic.

In our work, the susceptibility of the isolates to beta-lactam antibiotics, the aminopenicillins and cephalosporins was 75-100 %, being in line with the studies which reported 90-96 % susceptibility of *H. somni* to this group antibiotics in the USA and Canada [10-12]. Note, 25 % of the tested isolates were resistant only to aminopenicillins. One sample was resistant to the 3rd generation cephalosporin cefotaxime, while there was 100 % susceptibility to other 3rd generation drugs, the ceftazidime and ceftriaxone.

According to our data, the susceptibility of the *H. somni* isolates to antibiotics of the tetracyclines group, was also high, reaching 83-100 %. This is consistent with studies conducted in the United States, in which the susceptibility of *H. somni* to tetracycline was 88-100 % [10, 11]. Later, Portis et al. [12] reported that the susceptibility of *H. somni* to tetracycline has been steadily decreasing every year and has almost halved in 9 years, from 83 to 47 %. It should be noted that in another study, the authors reported for 92 % susceptibility of *H. somni* to tetracycline [15] which may be due to both differences in the sample size and the uneven distribution of resistant isolates in North America.

Among six isolates of *H. somni* we additionally tested for susceptibility to the sulfonamide antibiotics group sulphamethoxazole, 33 % were resistant. Approximately the same figures were obtained by Welsh et al. [11] who reported 68-86 % susceptibility to sulfachloropyridizine, also an antibiotic of this group. In an earlier work, more than half of the tested samples (64.2 %) were also shown to be resistant to sulfamethazine [10].

We identified two multi-resistant *H. somni* isolates possessing resistance to three classes of antibiotics, the aminoglycosides, beta-lactams, and tetracyclines. Four isolates showed phenotypic resistance to antibiotics of two classes, namely, two isolates were resistant to aminoglycosides and sulfonamides and two to aminoglycosides and beta-lactams. It should be noted that the multi-resistance of *H. somni* was revealed earlier when studying bacteria isolated from sick animals at feedlots in different states of the United States. In the work of American scientists, 30 % of the studied *H. somni* isolates were resistant to more than seven classes of antibiotics, including aminoglycosides, macrolides, tetracyclines, beta-lactams, fluoroquinolones, lincosamides, and pleuromutilins [25].

To detect genetic determinants of antimicrobial resistance by PCR test, we used the following primers (Table 2).

2. Primers used to detect genetic determinants of antimicrobial resistance in 18 isolates of *Histophilus somni*

Antibiotics	Target gene	Nucleotides 5′-3′	Tm, °C	Reference
Streptomycin/ spectinomycin	*AadA25*	5′-GGCAACGCTATGTTCTCTTGCTTTTG-3′ 5′-TGTACGGCTCCGCAGTGGA-3′	60	This work
Neomycin/ gentamicin	*StrA*	5′-GGCGGCTGATCTGTCTGG-3′ 5′-CAGATAGAAGGCAAGGCGTTC-3′	59	[25]
Neomycin/ gentamicin	*StrB*	5′-CGCGTTGCTCCTCTTCTCCA-3′ 5′-GGCTACATGGCGATCTGCATC-3′	60	[26]
Kanamycin/ neomycin	*aphA1*	5′-TTATGCCTCTTCCGACCATC-3′ 5′-GAGAAAATTCACCGAGGCAG-3′	55	[25]
Sulfonamides	*Sul2*	5′-CCAATACCGCCAGCCCGTCG-3′ 5′-TGCCTTGTCGCGTGGTGTGG-3′	64	[25]
Tetracyclines	*tetH*	5′-CCACCATTATGATCAGTATGTCT-3′ 5′-CATCAGCCATAACAGACCATC-3′	55	This work
Penicillins	*blaOXA-2*	5′-GCAGACGAACGCCAAGCGGA-3′ 5′-CCCGCACGATTGCCTCCCTC-3′	64	[25]

The genes for *H. somni* resistance to tetracyclines *tetH* and penicillins bla_{OXA-2} were not detected in any of the 18 isolates (Table 3). Genes for resistance to aminoglycosides, the *strA* and *strB* (44 % of samples), *aadA25* (39 %), and *aphA1* (11%) were the most common. The sulfonamide resistance gene *sul2* was

detected in 3 of 18 isolates. In contrast to our results, Stanford et al. [20] did not detect the *aadA25* gene in none of the 42 *H. somni* isolates. Neither we nor the authors identified the *bla*$_{OXA2}$ gene in any of the isolates [20]. In another work of Canadian scientists who investigated *H. somni* isolates for the presence of 13 genetic determinants of resistance and 5 genes associated with ICE, 26 % did not contain either ICE genes or resistance genes. The rest 74 % made two groups, one having six resistance genes and the other with nine resistance genes and, moreover, both groups had 4 genes associated with ICE [20].

3. Detection of antibiotic resistance genes in 18 isolates of *Histophilus somni* by PCR test

Group of antibiotics	Resistance gene	Enzyme encoded by resistance gene	Number (%) of positive samples
Aminoglycosides	*aadA25*	Aminoglycoside-3″-adenyltransferase	7 (39 %)
	strA	Aminoglycoside-3″-phosphotransferase	8 (44 %)
	strB	Aminoglycoside -6-phosphotransferase	8 (44 %)
	aphA1	Aminoglycoside -3′-phosphotransferase	2 (11 %)
Tetracyclines	*tetH*	Efflux protein	0
Beta-lactams	*bla*$_{OXA-2}$	Class D beta-lactamase	0
Sulfonamides	*sul2*	Dihydropteroate synthase	3 (17 %)

Whole-genome analysis of seven *H. somni* isolates [20] revealed the *strA*, *strB*, and *sul2* genes to mostly found. These results are consistent with our data on the identification of genes for resistance to aminoglycosides, however, unlike the work of Canadian scientists, we have never found the *tetH* gene. In two isolates with combined resistance to aminoglycosides, penicillins, and cephalosporins revealed in antimicrobial susceptibility testing by disk diffusion, only the genes for resistance to aminoglycosides *aadA25* and *strA* were detected. In two samples phenotyped as resistant to aminoglycosides and sulfonamides, almost all studied resistance genes were detected, except for *tetH* and *bla*$_{OXA-2}$. It is possible that the *strA*, *strB*, *aadA25*, *aphA1*, and *sul2* genes, like the resistance genes described for *H. somni* in other works [20, 25], are located in a mobile gene cassette which can move around within an organism's genome or be transferred to another organism. However, since we did not identify hereby the *tetH* gene, this gene cassette seems to differ from the ICEs previously described [14, 20, 25] and requires further study.

We compared the results that we obtained by microbiological and molecular methods (Table 4).

4. Correspondence of phenotypic and genotypic resistance in 12 *Histophilu somni* isolates

Group of antibiotics	Antibiotics	S phenotype		R phenotype		Correspondence, %
		R genotype	S genotype	R genotype	S genotype	
Beta-lactams	Ampicillin	0	9	0	3	75.0
	Amoxicillin	0	9	0	3	75.0
	Cefotaxime	0	11	0	1	91.7
	Ceftazidime	0	12	0	0	100
	Ceftriaxone	0	12	0	0	100
Tetracyclines	Tetracycline	0	12	0	0	100
	Doxycycline	0	10	0	2	83.3
Aminoglycosides	Streptomycin	0	6	6	0	100
	Neomycin	0	5	7[a]	0	100

N o t e. S — susceptible, R — resistant; [a] — includes isolates of intermediate resistance.

Phenotypic resistance to antibiotics and genetic determinants of resistance have been demonstrated for *H. somni* earlier [5, 20, 25]. By Stanford et al. [20], the correspondence between phenotypic and genotypic resistance for aminoglycosides was extremely high, up to 100 %, which coincides with our findings indicating the occurrence of genetic determinants of aminoglycosides resistance in all strains phenotypically resistant to these antimicrobials. In streptomycin-resistant strains the *aadA25* gene was found. In all five neomycin-resistant strains, the

presence of both *strA* and *strB* genes was detected. Nevertheless, in two strains with intermediate resistance to neomycin, the *strA* and *strB* genes were not detected, but the *aadA25* gene was found. Adenylation of neomycin appears to reduce the effectiveness of this antibiotic, but does not lead to absolute resistance. The *aphA1* gene was found in two samples resistant to both streptomycin and neomycin. Such a high correspondence between the pheno- and genotyping data will make it possible to predict the resistance of isolates to aminoglycosides by molecular methods without time-consuming microbiological procedures.

When comparing genotypic and phenotypic resistance to sulfanilamides, a good agreement was also obtained: in our work it was 100 %, Owen et al. [19] reported 94 %. Both our study and the report of the American authors [19] have shown a significant variability in the phenotype to genotype correspondence for resistance to beta-lactams and tetracyclines. In our study, the *bla*$_{OXA-2}$ gene was not detected in any of the three samples phenotypically resistant to penicillins and cephalosporins. According to the literature data, five different genes encoding beta-lactamases have been found in members of the *Pasteurellaceae* family. However, to date, only *bla*$_{OXA-2}$has been detected in *H. somni* [21], and, therefore, we performed genotyping of resistance to beta-lactam antibiotics only for this gene. It cannot be ruled out that other genes encoding beta-lactamases (*bla*$_{ROB-1}$, *bla*$_{CMY-2}$, *bla*$_{PSE-1}$, or *bla*$_{TEM-1}$) which can be transmitted to *H. somni* from *P. multocida* and *M. haemolytica* by plasmids and mobile genetic elements may occur in the three samples we found to be phenotypically resistant to beta-lactams.

The *tetH* gene which encodes a membrane-associated protein responsible for the active transport of tetracyclines outside the cell (efflux) was also not detected in the *H. somni* isolates we studied, despite the fact that two isolates showed resistance to doxycycline. Apparently, the resistance of these isolates is due to other mechanisms.

Thus, our findings showed *Histophilus somni* to be resistant to antibiotics of the aminoglycoside and sulfonamide groups but highly susceptible (over 90 %) to tetracyclines and 3rd generation cephalosporins, which allows assumption that tetracyclines and 3rd generation cephalosporins will be highly effective in the antibacterial therapy of diseases caused by *H. somni*. A high correspondence occurs between the data of microbiological and molecular detection of resistance to aminoglycosides and sulfonamides, and therefore, the PCR method can be recommended to predict the resistance of *H. somni* to the antimicrobials of these groups. Given published data on the presence of other genes for resistance to macrolides and florfenicol *erm(42)*, *mrs(E)-mph(E)*, and *floR*, and due to the wide use of antibiotics of these groups in the Russian Federation, we believe that the study is antibiotic resistance of *H. somni* must be continued.

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