FERTILIZATION FAILURE IN HEIFERS INFECTED BY UREAPLASMA DIVERSUM

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Ureaplasma diversum is an opportunistic pathogen in cattle, but colonization of the respiratory tract by this ureaplasma, and its carriage in the reproductive tract may lead to the serious diseases. It also may be the cause of abortion and stillbirth in cattle.

The aim of this study was to estimate the fertilization effectiveness of in heifers into relation with U. diversum carriage in the vulval vestibule. All 20 heifers in the study group were from the same dairy farm from Leningradskaya oblast. The samples were collected from the vulval vestibule by cotton swab. At the sample collection vulvar mucous appearance was estimated. The U. diversum carriage was detected by real-time PCR assay with diagnostic system “Ureaplasma diversum Amp” (St. Petersburg Institut Pasteur, Russia).

In the group of 20 heifers, 13 had symptoms or granular vulvovaginitis, including yellowish-gray pustules on the mucous. No other reproductive disease symptoms were detected in any heifer. The carriage of U. diversum was detected in 13 of animals. The granular vulvovaginitis is commonly associated with U. diversum carriage in heifers and cows, but the symptoms of this disease are nonspecific and frequently may be associated with other diseases, for example with bovine rhinotracheitis, that is very widespread in cattle. No association was detected between granular vulvovaginitis symptoms and U. diversum carriage in study population.

The effectiveness of fertilization was estimated in all heifers. The average number of inseminations leads to fertilization in heifers without carriage was 1.2, but in infected heifers it was 1.9, and the difference between two groups was statistically significant (t = 0.36; p < 0.002). The fertilization failure was more frequent in heifers with U. diversum carriage. Twelve heifers from this group were fertilised at first insemination, two heifers in the same group were fertilized at second insemination and one heifer was inseminated six times before fertilization. Into the group without U. diversum carriage all but one of heifers were fertilized in the first insemination an one heifer in second insemination.

The loss in fertilization effectiveness leads to the economic burden in dairy farms due to costs of repeated inseminations and animal management. The appropriate diagnosis of U. diversum carriage in heifest may improve dairy farm productivity.

ANTIBIOTIC-RESISTANT KLEBSIELLA PNEUMONIAE IN THE GUT MICROBIOTA OF HEALTHY INDIVIDUALS

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Klebsiella pneumoniae causes a wide range of infectious diseases including pneumonia, urinary tract infections, bacteremia and liver abscesses. Previously, it was believed that K. pneumoniae can cause serious infections primarily in people with decreased immunity, but the recent emergence and spread of hypervirulent strains has increased the number of people susceptible to these infections, including healthy ones. In addition, strains of K. pneumoniae are becoming more resistant to antibiotics, which creates special difficulties in treatment. Strains of the genus Klebsiella quite often colonize the mucous membrane of the distal gastrointestinal tract of children and adults, being part of the gut microbiota.

The aim of this work was to study the quinolone and β-lactam resistance mechanisms in Salmonella and opportunist bacteria strains isolated from farm animals.

We determined the quinolones and β-lactams susceptibility and resistance mechanisms in 482 Salmonella and 144 strains of opportunistic bacteria (E. coli, Klebsiella spp.).

For 6 Salmonella strains (3 S. Enteritidis and 3 S. Infantis), resistant to fluoroquinolones the mutations in the QRDR region of gyrA gene were detected by amplification and sequencing of this DNA region (Kosyrev et al., 2012). The extended-spectrum β-lactamases (molecular classes A and C) were determined by PCR with specific primers (Dallenne et al., 2010). 132 strains of Salmonella were resistant to quinolones (27.4%), 41 of them (8.5%) had high level resistance to ciprofloxacin (MIC 6–32.0 mg/l). Sequencing of the gyrA of some resistant Salmonella isolates have been identified three types of single point mutations. In two S. Enteritidis the mutation was noted in 83 position (Serine replace-ment by Phenylalanine), in one strain — in 87 position (Asparagine replacement by Glycine). Three S. Infantis strains had the replacement of Asparagine by Tyrosine in 87 position.
9 strains of *Salmonella* (1.9%) were resistant to extended-spectrum cephalosporins. According the beta-lactamase inhibitor susceptibility tests, five of them were classified as ESBL-producers, 4 strains — as AmpC-producers. ESBL CTX-M was detected in strains of *S*. Haifa isolated from chicken samples and *S*. Derby isolated from the imported pork heart. AmpC cephalosporinase CMY was produced by two strains of *S*. Kentucky, isolated in 2006 and 2009 from imported poultry products, as well as two *S*. Dublin isolated in 2005 from the internal organs of a fallen calf and cow.

In opportunistic bacteria (*E*. coli and *Klebsiella* spp.) 22 strains were resistant to extended spectrum cephalosporins, 15 of them produced ESBL according the beta-lactamase inhibitor susceptibility tests. The class of detected β-lactamases was established in 11 strains. In *K. pneumoniae* and *K. ozanae* isolated from the milk of cows sick with mastitis ESBL CTX-M1 were detected. In *E. coli* isolated from calves suffering from diarrhea, was detected ESBL.CTX-M1 and CTX-M9. So, our study has confirmed the circulation of *Salmonella* and other *Enterobacteriaceae* strains resistant to clinical significant antibiotics (fluoroquinolones and cephalosporines) in animal farms.

The study’s aim was the data analysis of the *K. pneumoniae* mass spectra for protein biomarker discovery that make it possible to predict the detection of strains with OXA-48 and NDM-1 carbapenemase activity.

We used archived spectra obtained for the routine identification of isolates from hospital patients of St. Petersburg in 2015—2017. Digital data of 67 raw spectra, selected by identification results at the *K. pneumoniae* species level, were exported to the “BioNumerics” software. The created classifier was used to identify of new seven OXA-48 and eight NDM-1 strains pre-characterized by PCR, and 16 sensitive to meropenem *K. pneumoniae* strains. The biomarker peaks were designated by comparing their molecular weights with the data of plasmid proteins *K. pneumoniae* in the NCBI and UniProtKB bases with using the ExPaSy portal.

The cluster analysis results of 67 spectra were used to create a model, that consist of six classes. The aggregate efficiency of the classifier was 89.6%. The spectra of group #4 had a marker peak m/z = 5996 Da, which was comparable in molecular weight to the protein pKF140-142 of plasmid pKF3-140. The marker peak m/z = 6096 of group #2 was designated as a plasmid protein according it coincidence on molecular weight with the protein UUU_02980 of plasmid pKPt2. Sixteen sensitive strains were mainly classified in group #2, but their spectra lacked a plasmid peak m/z = 6096. Eight NDM-1 strains were assigned to different groups, however their spectra showed either the peak of the plasmid protein m/z = 6096 or the peak m/z = 5936 which was also identified as a plasmid protein according to the protein of the outer membrane receptor protein of the plasmid pF77. All OXA-48 strains were assigned to group #4 and their spectra contained the peak of the plasmid protein m/z = 5996.

It has been suggested that the mass spectra of carbapenem-resistant *K. pneumoniae* strains may contain peaks attributed to the plasmid-encoded proteins. Such small plasmid proteins, which molecular weight don’t correspond to the carbapenemases, even so, can appear as predict biomarkers of carbapenemase activity of strains.