

58m strain (GenBank Acc. No. LGAN00000000) genome was detected. Also blaOXA-335 beta-lactamase gene was identified in the genome of the strain *A. Iwoffii* 51m (Acc. No. LZDF00000000) from mammoth intestinal tract (Goncharov A. et al., 2016). Based on this data we believe that the formation of epidemic clones in opportunistic bacteria is more likely determined by the natural selection of strains that carry genetic determinants of pathogenicity or resistance to antimicrobial drugs from natural populations (including in the polar regions of our planet), than by their formation in human society *de novo*.

2.6

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THE RESULTS OF THE RESEARCH OF USING A COMMERCIAL KIT FOR DETECTION THE RABIES VIRUS'S RNA IN THE COURSE OF EVALUATION OF THE INFECTION OF THE FIELD MATERIAL

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There are 3000 animal diseases of the Rabies registered annually in Russia (Poleshchuk et al., 2013). The monitoring of this disease helps to reduce it for animals and people. Forward-looking molecular genetic methods are used more and more often in order to diagnose the disease (Iovleva et al., 2012; Devyatkin et al., 2014; Dedkov et al., 2016).

"The kit for the detection of the Rabies virus's RNA in a complete set" produced by Ltd "Fractal Bio" (St. Petersburg) is a set of reagents for the outflow of the nucleic acid and the realization of the real-time PCR with hybridization-fluorescent registration, it was used to analyze the Rabies of 210 samples: 153 field material and 57 samples of bioprobes.

The initial material included samples of foxes, raccoon dogs, corsac foxes, minks, Siberian striped weasels, martens, ferrets, sables, ermines, bats and men as a 10% suspension of a cerebrum based on Henx solution. There were also the samples of bioprobes of not purebred white mice that died in the course of the experiment. The samples were collected from 2–11 passages and were presented by 10% suspensions of the cerebrum. The apparatus used during the research was RotorGene6000.

With the use of test-system, the presence of Rabies virus's RNA was confirmed in all positive samples of the initial material of foxes (n = 14), cattle (n = 6) and men (n = 2). None was detected in all the negative samples. If negative probes, they registered the formation in the reaction of the product of the amplification of the internal control sample (genomic DNA of mammals).

A different selection of the field material revealed drawbacks of the set when using the internal control sample (ICS). Therefore, ICS did not work with the initial samples of raccoon dogs (n = 10), bats (n = 12) and all the mice samples. When researching the initial material of foxes (n = 35) and corsac foxes (n = 3), there was no reaction of the ICS or it was very weak (from the 30th – 36th cycle) in more than 50% cases. The dilute of the nucleic acid, repeated several times, did not improve the result of the reaction with the ICS. That means it lacks the universality of the amplifiable fragment. Provided that there is high reliability of identification of the Rabies virus's RNA, the negative results of the ICS make the interpretation of the result more difficult.

Therefore, the above-mentioned test-system needs refining in part of the optimization of the ICS.

2.7

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COLLISION OF CRISPR-CAS SYSTEMS WITH THE POTENTIAL OF VIRULENCE OF *ESCHERICHIA COLI* STRAINS THAT PRODUCE SHIGA-TOXINS

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Shiga toxin-producing *Escherichia coli* (STEC) strains are a diverse group of *E. coli* strains belonging to over 400 *E. coli* serotypes, some of which cause outbreaks and sporadic cases of food-borne illnesses ranging from diarrhea to hemorrhagic colitis and the hemolytic-uremic syndrome (HUS). It was long believed that bacteria could not resist phage attacks, but in 1987 a strange region was discovered in the *E. coli* genome that consisted of multiple repeats. The discovered structures were termed CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats — CRISPR-associated proteins). CRISPR is a chronological record of infectious assault on a bacterium from viral and other genetic elements. According to Delannoy S. et al., It has been shown that CRISPR polymorphisms in *E. coli* strongly correlate with both the serotype of the microorganism and the presence of virulence factors in its genome (*stx* and *eae* genes).

Genomic sequences (GenBank databases) of *E. coli* isolates of different serogroups (n = 658) were analyzed for the presence of CRISPR-Cas systems and *stx*-genes. Of the 658 *E. coli* isolates, 60.5% of the loci of CRISPR-Cas systems were found. At the same time, according to the structural organization of CRISPR-Cas, the systems of the strains studied were of type I-E in 92.7% and type I-F of 7.3%. Analysis for the presence of genes of shiga toxins 1 and 2 types showed that 14.4% of isolates having a CRISPR-Cas system of type I-E were positive. The genes *stx1A* and *stx1B* were registered in 6.1 and 5.9% of cases. The frequency of registration of *stx2* subtypes was 2 times higher than *stx1* (6.1 and 12.3%). *Stx2B* was detected in 9.9% of cases.

Stx2A, which according to the literature is more often associated with HUS than other subtypes, was detected in 9.7%. According to the CRISPR-Cas data, the I-E subtype system is associated with the *stx1A* (10.1%), *stx1B* (9.8%) *stx2A* (16.1%) and *stx2B* (16.3%) genes. Further linkage between CRISPR elements and the pathogenicity of the isolate will allow us to determine the causal relationships that stimulate the acquisition of the isolate of both the CRISPR-Cas system and the genes coding for pathogenicity.

2.8

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NGS CAPABILITIES FOR THE STUDY OF ENTEROAGGREGATIVE *E. COLI*

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Enterotoaggregative *E. coli* (EAggEC) are the causative agents of such intestinal diseases as acute and chronic diarrhea, inflammation of the intestine in children and