

RESEARCH ARTICLE

Pollution Monitoring in The Protected Areas to Ensure Safety in The Kyrgyz Republic

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ABSTRACT

The given article provides preliminary outcomes of researches on monitoring of Pas in the Kyrgyz Republic on the availability of the agents of natural and focal diseases: anthrax, tularemia, leptospirosis and brucellosis. The article also considers the risk of infection of tourists and other visitors in the Kyrgyz Republic.

KEYWORDS:

biosafety, monitoring, protected areas (PAs), socially significant zoonotic diseases. Arboviruses, anthrax, tularemia, leptospirosis, brucellosis.

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INTRODUCTION

According to the forecasts of the World Tourism Organization by 2020 the Great Silk Road will be the most popular tourist route in the world. High mountains, glaciers, blue lakes, noisy rivers, alpine meadows rich in flowers make up the Kyrgyz part of the Great Silk Road.

The Program of the Kyrgyz Republic Government for 2019-2023 reflects current global trends in Tourism sector and the current situation in the tourism industry of the country.

In accordance with the Kyrgyz Republic Presidential Decree the relevance of regional development is aimed, first of all, at improving the living standards of the population and realizing

opportunities for sustainability and reproduction of the environment.

The foregoing national programs for the development of the country require the study of biological risks on tourist routes ensuring biological safety of tourists and other visitors to PAs.

MATERIALS AND RESEARCH METHODS

Sampling and extraction of genomic DNA

In 2017-2019 there were 17 expeditions on the territory of the Kyrgyz Republic to collect biomaterials from the fauna for further investigation on the presence of pathogens. The scientists of Biotechnology Institute, National Academy of Sciences of the Kyrgyz Republic selected more than 1000 specimen in triple replication from each specimen (Fig.1).

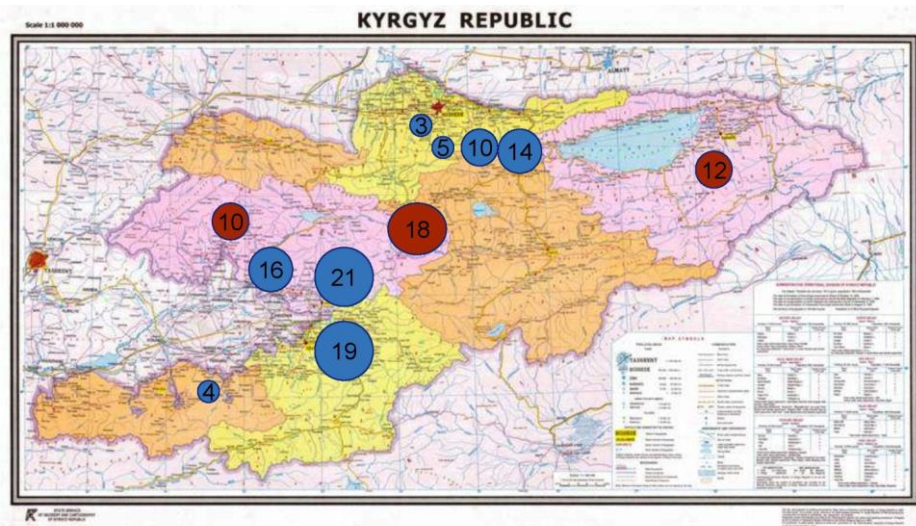


Fig.1: Map of sampling in 2017

Laboratory works were carried out at Biotechnology & Nutrition Lab of Biotechnology Institute, National Academy of Sciences of the Kyrgyz Republic in aseptic conditions. The transported biological samples were preserved in a freezer at - 80°C. Prior to extraction of genomic DNA the samples were moved on a tray and defrosted at a room temperature. Genomic DNA was extracted from internal organs: liver, spleen, lungs taken from each biological specimen. Extraction of genomic DNA was done according to Protocol of QIAamp DNA Mini Kit (Castella, V., et al. 2006), 25mg of tissue previously reduced was used. Extracted DNA was placed in a freezer at -200C till the analysis.

Checking DNA quality

Quantitative and qualitative assessment of extracted DNA was done on spectrophotometer NanoDrop 2000 (Thermo Scientific) and through gel-electrophoresis. 1,5% of agarose gel with Tris-acetate buffer 1 X TAE was employed.

Visualization was implemented through adding a dye of ethidium bromide. Total volume of mixture made up 6 µcl,

consisting of 2 µcl, DNA Gel Loading Dye (6X)+ 4 µcl of genomic DNA. To rate DNA sizes DNA bar 100 base pairs (Sigma Aldrich). The gel was run for 30min. at 80 V and detected in UV-light on trans illuminator TCP-20 MS and CCTV camera.

qPCR

PCR real time was implemented on platform StepOnePlus Real-Time PCR System, Applied Biosystems on the availability of Bacillus anthracis, Brucella spp, Francisella tularensis, Leptospirosis spp. In samples. Kits for detection - TaqMan Bacillus anthracis Detection Kit, TaqMan Francisella tularensis Detection Kit were employed according to the guidelines of Applied Biosystems manufacturer (Malcolm, et al., 2000).

Preparation of libraries and sequencing

Duplicates of the samples were shipped to Institut de Recherche Biomédicale des Armées (IRBA). The preparation of library included several steps (Fig.2). Sequencing was run on platform Illumina HiSeq according to Protocol of Company-Manufacturer.

Library preparation

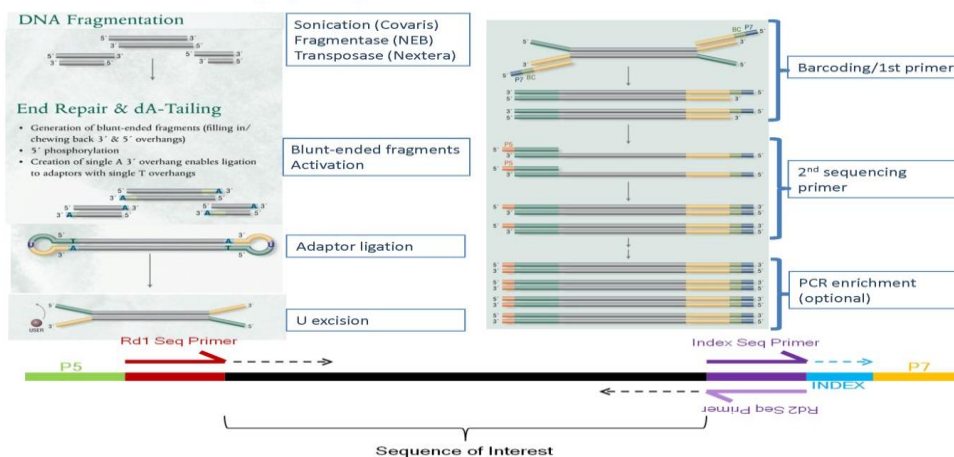


Fig.2: Preparation of library

When processing the data, the following programs were used: FastQC (Brown et al., 2017), cutadapt (Marscel, 2011), Mapping reads: Kraken2 (Wood et al., 2019) (The Center for Computational Biology at Johns Hopkins University), Visualizing mapped reads: krona (Nur A., et al, 2014).

Research outcomes

Over the past 10 years the scientists of Biotechnology Institute, National Academy of Sciences of the Kyrgyz Republic carry out purposeful monitoring on the assessment of biological hazards. These studies in addition to basic researches are of great importance for practice.

Biotechnology Institute, National Academy of Sciences of the Kyrgyz Republic studied the patterns of spatial distribution of natural foci of anthrax. An electronic cadaster and computer database of bad foci located on the territory of Kyrgyz Republic were created. These scientific developments were transferred to Ministries of Agriculture and Processing Industry, Ministry of Health, Ministry of Emergency, Transport etc. for practical use.

The regular connection of the Great Silk Road to the contamination of the territory of the Kyrgyz Republic by the causative agent was established. (Fig.3)

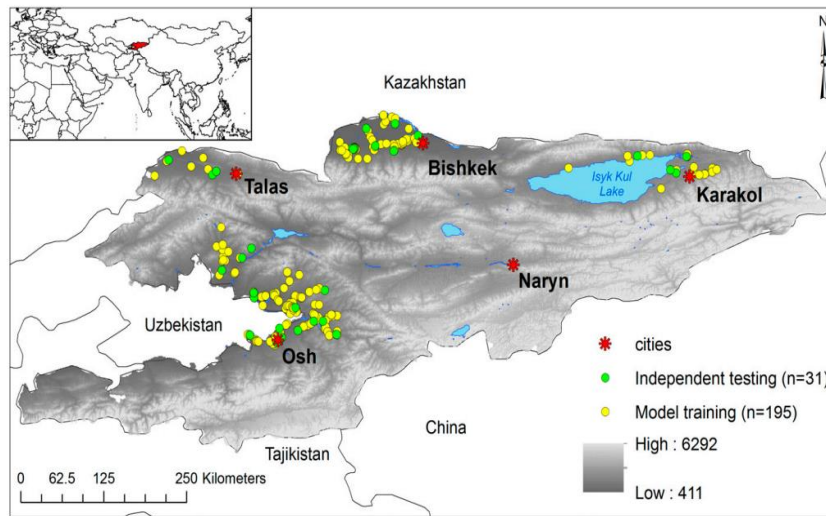


Fig.3: Stationary anthrax foci on caravan routes of the Great Silk Road

Jointly with the scientists from Center for Quarantine, Especially Dangerous Infections and Karakol University after K.Tynystanov the natural foci of plague and arbovirus

infections were studied. The patterns of distribution of these infections' foci on caravan route of the Great Silk Road were determined. They are given in Figs.4 and 5

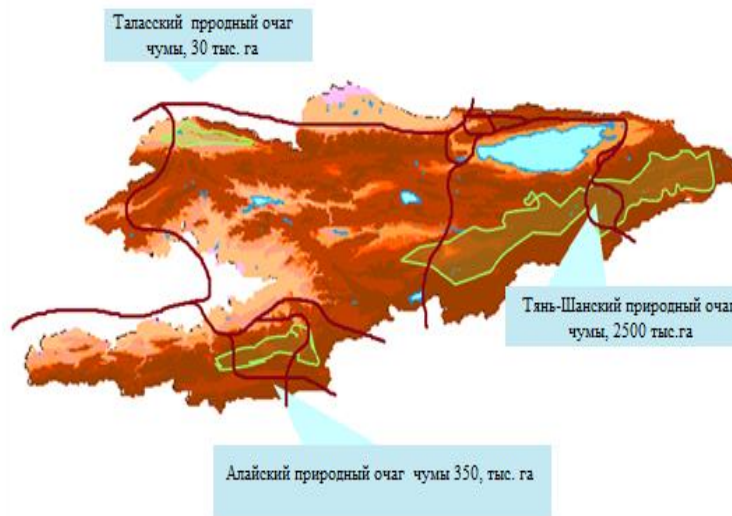


Fig.4: Natural foci of plague on caravan routes of the Great Silk Road



Fig. 5: Natural foci of arboviruses on caravan routes of the Great Silk Road

In order to avoid any hazards of contamination and transfer of infections on other territories, the scientists of Biotechnology Institute, National Academy of Sciences of the Kyrgyz Republic jointly with Institut de Recherche Biomédicale des Armées (IRBA) carried out for three years researches to

monitor the presence of the pathogens of socially significant diseases: anthrax, tularemia, leptospirosis and brucellosis.

A small forest mouse (*Apodemus uralensi*) was the most frequently found species in sampling places in 2018. This mouse species is a carrier of agent of brucellosis, tularemia, anthrax and other diseases (Fig.6).

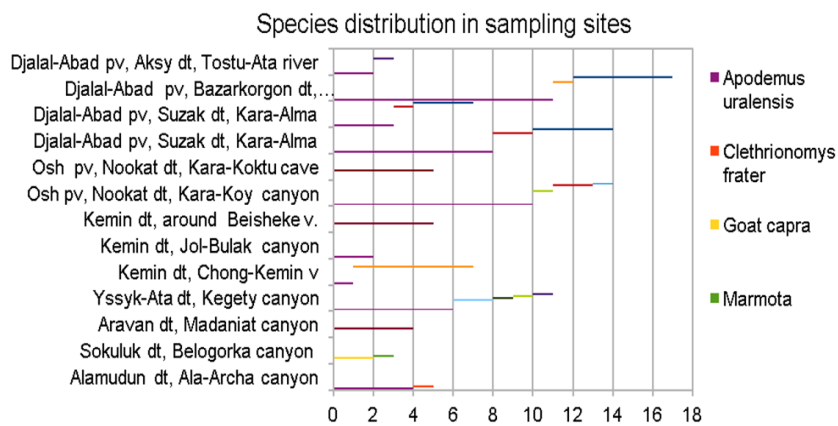


Fig.6: Distribution of wild animals in biomaterial sampling places in 2018

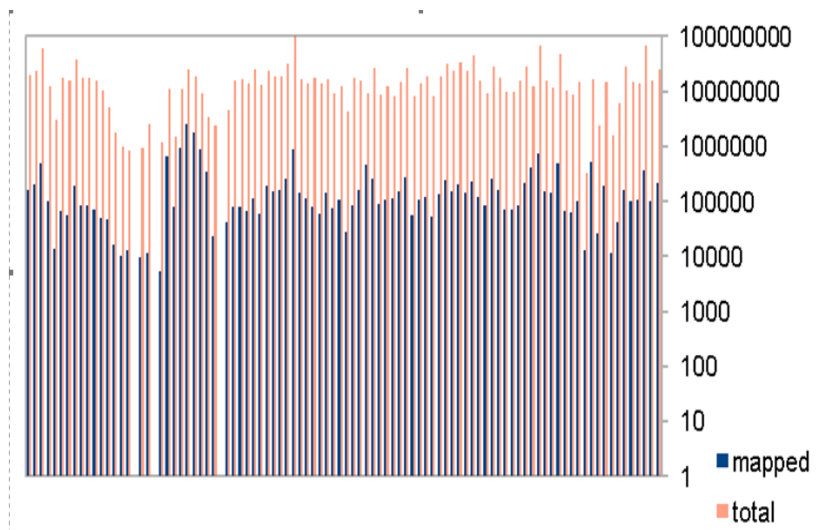


Fig. 7: Sequencing results of totally obtained reads (sites); red column, from them read - dark blue diagram

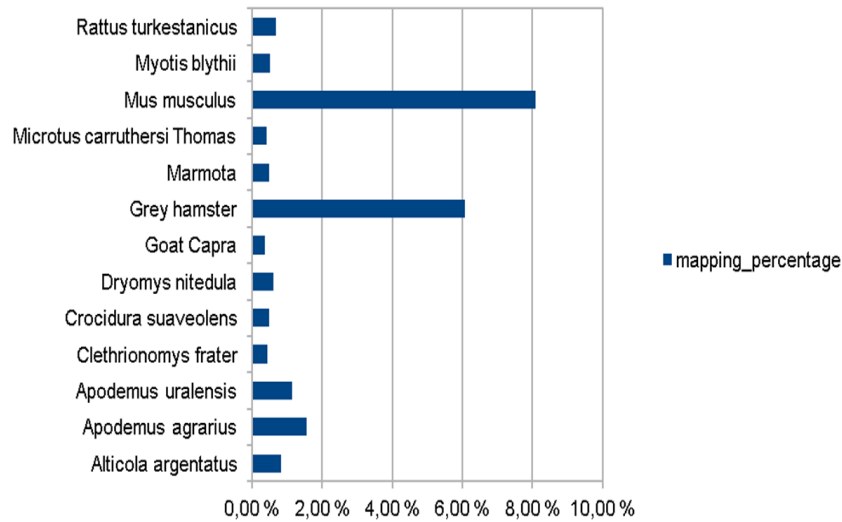


Fig.8: Percentage ratio of mapping from obtained reads by wild animal species

On Figure 8 it is seen that in our studies animal species has their effect on percentage of reads, which can be used when mapping.

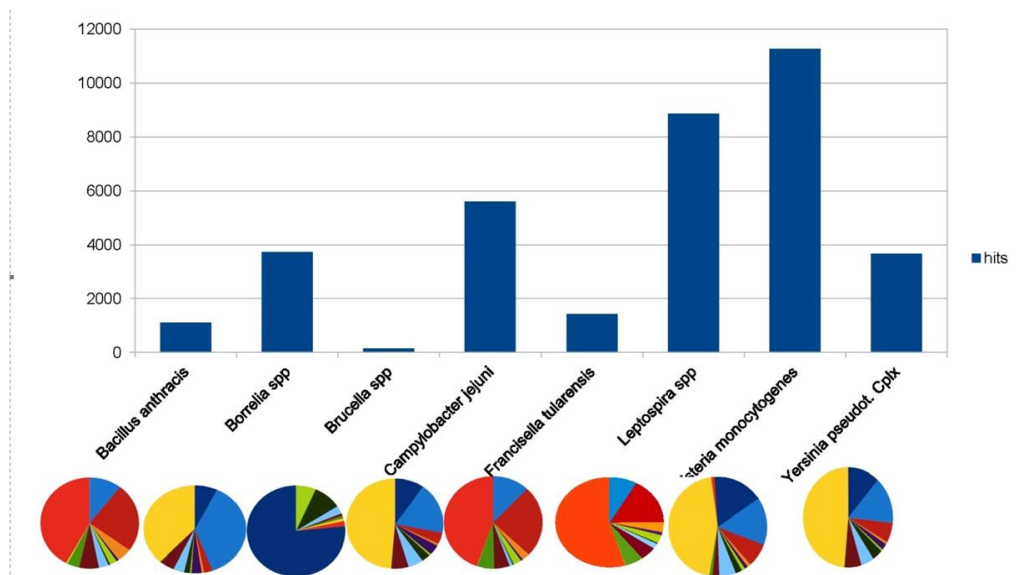


Fig.9: Preliminary result on presence of bacterial pathogens in the studying samples (numerical scale shows a number of concordances on the presence of bacterium)

Processing through programs of the obtained reads showed, that in the studying samples, there might be zoonotic pathogens, including especially hazardous. However, this is preliminary data. Total 96 libraries which were employed for sequencing have been prepared. When sequencing, about 100 million reads (sites) were obtained, which will be processed through bioinformatics programs. Samples with positive signals have been transferred for repeated sequencing, which will be completed at the end of the current year.

CONCLUSION

Monitoring potential places in the regions of the Kyrgyz Republic on the availability of socially significant diseases through up-to-date techniques for biotechnology and molecular genetics is urgent and necessary for the population to prevent diseases risk. The gathered rich genetic material is preserved in proper conditions at the Biotechnology Institute, National Academy of Sciences of the Kyrgyz Republic for

further thorough study. Parallel work on diseases caused by viruses, i.e. RNA sequencing is scheduled. When finalizing the outcomes the areas and wild animal species which are of potential hazard of transfer of zoonotic diseases on new territories will be determined.

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