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Research Article

Assessment of the Spread of Certain Pathogens Among Laboratory Rodents at Universities and Academic Institutes of Almaty, Kazakhstan

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Abstract | The health of laboratory animals is one of the key components of ensuring the quality of research results. Microbiological status uncertainty creates risks not only for scientific research, but can also pose a danger to the researchers themselves. In this paper we present the results of the assessment of microbiological quality of laboratory animals from some universities and academic institutes in Almaty city, Kazakhstan. This is the first time that such work, involving several institutes, has been carried out. To do this, several pathogenic bacteria and viruses from the Federation of European Laboratory Animal Science Associations (FELASA) were selected. The main methods of testing biological samples from laboratory mice and rats were realtime polymerase chain reaction and enzyme-linked immunosorbent assay. A high prevalence of some pathogens among conventional laboratory animals used by some academic institutes and universities of Almaty has been shown. *Rodentibacter pneumotropicus* has been detected in the SPF animals, while no other pathogens from the FELASA list have been detected. The obtained results show the importance of not only regular health monitoring, but also the need to improve the quality of care and well-being of laboratory animals in some facilities of the Almaty city.

Keywords | Health monitoring, Mice, Rats, Rodent pathogens, Specific-pathogen free

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INTRODUCTION

Among laboratory animals, mice and rats are the most common species used in scientific research. However, it is difficult to estimate the number of their use and the level of well-being in some countries (Kim et al., 2017; Rydell-Törmänen and Johnson, 2019; Carbone, 2021). Their anatomy, physiology, behavior, health, biological needs etc. are well studied (Frohlich, 2020). It should also be noted that the care conditions and the quality of the used laboratory animals affect the research results. For example, enriching the habitat and ensuring the natural behavior of rodents improves the research results (Bayne and Würbel, 2014; Cait et al., 2022). Another factor ensuring the research quality is preservation of the laboratory animal health (Fahey and Olekszak, 2015). For that purpose, recommendations for monitoring the laboratory animal health have been developed by the Federation of European Laboratory Animal Science Associations (FELASA) (FELASA et al., 2014). Studies of the quality and prevalence of infections among laboratory animals are regularly conducted and reports are published in different countries

(Seok et al., 2005; Manjunath et al., 2015; Rodrigues et al., 2017). For example, it is noted that the quality of laboratory animals from manufacturers is higher than from academic institutes (Pritchett-Corning et al., 2014). There are different methods of testing for pathogens, depending on the type of pathogen, the prevalence among the animal colony, the sensitivity, type and quality of the tested samples (Henderson et al., 2013; Compton, 2020). Therefore, the goal of this research was to study the spread of some pathogens among laboratory mice and rats from universities, academic institutes and one SPF animal manufacturer in Almaty. The National Scientific Center of Especially Dangerous Infections (NSCEDI) was reconstructed under the ISTC and EU project in 2014. SPF animals were received from Envigo+ (Netherlands) in the same year. One of the conditions was saving animals' lives after sampling. For this research it was acceptable to use realtime polymerase chain reaction (rt-PCR) for testing of samples of feces and mouth swabs (Henderson et al., 2013). In addition, blood sera of mice and rats for antibodies to viruses was examined by using the ELISA method as part of the annual health monitoring in NSCEDI (Manjunath et al., 2015).

MATERIALS AND METHODS

Ethical Statement

The study protocol was reviewed and approved at the IACUC meeting of the National Scientific Center of Especially Dangerous Infections (No. 86/2 dated March 17, 2020).

Sample collection

Samples of feces and mouth swabs were obtained from adult mice (n = 10) and rats (n = 10) aged 2.5-3 months from the vivaria of three universities, two academic institutes and from one SPF animal manufacturer (NSCEDI). Samples were delivered and tested on the same day. The animals were not killed and remained in the facility.

Serum (500 µL) from mice and rats from NSCEDI was collected after inhalation euthanasia with 70% carbon dioxide.

DNA extraction for rt-PCR assay

500 µL of the Inhibitex Buffer (Qiagen, Netherlands) was added to each fecal sample and mouth swab and incubated for 15 minutes at 70 ˚ C (Sanyo, Japan). DNA isolation was performed according to the kit protocol (QIAamp® DNA Mini Kit, USA). The DNA concentration was determined on the Qubit 4 fluorometer (Thermo Fisher Scientific, USA) using reagents (Thermo Fisher Scientific, USA). The DNA concentration in the samples ranged from 1.17 to 33.4 ng per µL.

rt-PCR assay

The *Clostridium piliforme*, *Helicobacter* spp, *Mycoplasma pulmonis*, *Streptococcus pneumoniae*, *Streptococcus b-haemolyticus*, *Rodentibacter pneumotropicus* (formerly known as *Pasteurella pneumotropica* (Adhikary et al., 2017), Mouse parvovirus NS1, Mouse parvovirus VP2, Minute virus of mice, Kilham rat virus and Rat parvovirus VP2 (Belki-Biotechnologies, Novosibirsk, Russia) kits were used in the rt-PCR. DNA amplification was carried out with 20 µL of the reaction mixture. The kit positive control (Belki-Biotechnologies, Novosibirsk, Russia) was used. Purified water served as a negative control. Amplification program: 5 min pre-incubation at 95˚ C followed by 40 cycles of 15 sec at 95˚ C, 25 sec at 62˚ C, and the luminosity readout for 25 sec at 62˚ C. Melting curve plotting was at temperatures from 65˚ C to 95˚ C with a step of 0.5˚ C in 5 seconds.

ELISA

Serum samples were analyzed for antibodies to the following viruses: Mouse Hepatitis Virus, Mouse rotavirus (EDIM), Theiler's murine encephalomyelitis virus and Murine norovirus – mice; and Rat minute virus, Pneumonia virus of mice, Rat coronavirus, Rat Theilovirus – rats. Positive and negative controls were used from the kits. The study was carried out accord ing to the manufacturer's instructions (BBTLAB, Russia), optical density was measured on a Wellwash AC plate reader (Thermo Fisher, USA).

Data analysis

The data collected from the study were analyzed using Graph pad Prism software (GraphPad Prism version 6.0, San Diego, CA, USA). For positive outcomes, a 95% confidence interval was estimated at p 0.05 (Hazra, 2017).

RESULTS AND DISCUSSION

Study on the health of laboratory animals in academic institutions and universities in Kazakhstan is being conducted for the first time. Samples for the study came from the vivaria of three universities, two academic institutes and one SPF animal manufacturer. Minimal information on animal care conditions was collected and summarized in Table 1.

Unfortunately, not all academic institutes and universities in Almaty keep laboratory animals in individually ventilated cages (IVC). Open-air cages (OAC) are practiced at one university and one institute, and animal feeding is carried out using natural feeds (grain, milk protein and feed additives). Preferred species and the microbiological status of laboratory animals also differ, and often lower quality animals are chosen.

Table 1: Care conditions of laboratory rodents in academic institutes, universities and SPF animal manufacturer in Almaty city

Facility	Animals and health status	Care condition	Diet
University 1	Mice and rats, conventional	IVC	Standardized and natural feed
University 2	Mice and rats, conventional	OAC	Natural feed
University 3	Mice, SPF	IVC	Standardized feed
Institute 1	Rats, conventional	OAC	Natural feed
Institute 2	Mice and rats, SPF	IVC	Standardized feed
Manufacturer (NSCEDI)	Mice and rats, SPF	IVC	Standardized feed

IVC - individually ventilated cage; OAC – open-air cage.

The prevalence of various mice and rats pathogens in these institutions of Almaty was also analyzed. To do this, FELASA recommendations for assess the microbiological status and health of animals were used, and several pathogens from the FELASA list were selected for which test kits were available (FELASA et al., 2014). We believe that the identification of even a few pathogens from the FELASA list can show the level of spread of pathogenic microorganisms among animals kept at Almaty facilities. Table 2 shows the sample test results for bacterial infections and Table 3 for viral pathogens.

Universities and institutes keeping conventional mice and rats have shown a high prevalence of both bacterial and viral pathogens. And regardless of the care method, OAC or IVC. For them, all tested samples were positive. On the contrary, wide data confidence interval for University 3 and manufacturer NSCEDI (95% C. I. 1-7 and 3-9, respectively) indicates a low accuracy in assessing the prevalence of pathogens in the population (Hazra, 2017).

The most common causative agent was *R. pneumotropicus*. This bacterium is an opportunistic pathogen that is dangerous especially for atypical immunodeficient mice. Immunocompetent mice are vectors, and infection has no clinical manifestations (Kawamoto et al., 2011). *R. pneumotropicus* can be transmitted through direct contact between animals, but is poorly transmitted through contaminated bedding (Scharmann and Heller, 2001; de Bruin et al., 2016). Unlike another pathogen *M. pulmonis*, which can be transmitted both vertically *in utero* and horizontally through a contact or an aerosol (Otto et al., 2015). Therefore, detection of *R. pneumotropicus* is unlikely to be associated with poor hygiene at NSCEDI and University 3. It is also known that *M. pulmonis* is detected more often than *R. pneumotropicus* among mice and rats from academic, industrial and government institutes in various countries (McInnes et al., 2011; Hansen et al., 2019). Therefore, it can be assumed that a small number of mice was initially contaminated by *R. pneumotropicus,* and the infection spread inside the NSCEDI colony as a result of animal breeding. This is confirmed by detection of *R. pneumotropicus* during regular monitoring of animal health at NSCEDI. Another common pathogen is the group of bacteria *Helicobacter spp*. It should be noted that *S. pneumoniae*, *S. b-haemolyticus* and *C. piliforme* were not found in the samples. However, their prevalence in animal colonies, as a rule, is not great (Pritchett-Corning et al., 2009).

It is obvious that conventional laboratory animals have a high prevalence of pathogens (Manjunath et al., 2015; Carriquiriborde et al., 2020). This cannot but raise concerns about the conditions of animal care and well-being. I would like to note that the results were shared with the staff of a particular facility, where the tests turned out to be positive. RT-PCR method has shown high efficiency in screening for pathogenic microorganisms, due to its high sensitivity, and allows performing tests without killing the animal (Henderson et al., 2013; Compton, 2020). Therefore, we did not use the so-called sentinel animals, but the pathogens were determined directly from live animals from the colony. This is also important because it is not always possible to identify all pathogenic microorganisms in animals using the technology for soiled-bedding sentinel animals (Mailhiot et al., 2020).

Combining individual ELISA with PCR allows you to more fully assess the health status of animals. However, the ELISA requires sufficient serum. This method is suitable for routine pathogen prevalence studies. This approach has been used in the only SPF animal manufacturer in Kazakhstan, where animal health is monitored quarterly. Table 4 shows the test results of blood sera from mice and rats for antibodies to some viral pathogens.

All tested serum samples from mice and rats from NSCEDI were negative for some viral pathogens significant in health monitoring (FELASA et al., 2014).

Perhaps due to the low prevalence of these viral pathogens, the probability of their detection was not high in SPF animal colonies, with the exception of Murine norovirus. The prevalence of this virus is 32%. However, it is not always possible to identify by ELISA (Pritchett-Corning et al., 2009).

Table 2: Prevalence of bacterial pathogens among mice and rats of vivarium institutes in Almaty city diagnosed by using rt-PCR

Facility	Mice		Rats	
	No. positive / Total No. (C.I.)	Pathogens*	No. positive / Total No.	Pathogens
University 1	10/10	*R. pneumotropicus,* and *Helicobacter* spp.	10/10	*R. pneumotropicus*, *M. pulmonis* and *Helicobacter* spp.
University 2	10/10	*R. pneumotropicus*, *M. pulmonis* and *Helicobacter* spp.	10/10	*R. pneumotropicus*, *M. pulmonis* and *Helicobacter* spp.
University 3	4/10 (1-7)	R. pneumotropicus	-	-
Institute 1	10/10	*R. pneumotropicus*, *M. pulmonis* and *Helicobacter* spp.	10/10	*R. pneumotropicus*, *M. pulmonis* and *Helicobacter* spp.
Institute 2	0/10	no	0/10	No
Manufacturer (NSCEDI)	6/10 (3-9)	*R. pneumotropicus*	0/10	no

C.I. - confidence interval; *Rodentibacter pneumotropicus, Helicobacter spp, Clostridium piliforme, Mycoplasma pulmonis, Streptococcus pneumoniae, Streptococcus b-haemolyticus,

Table 3: Prevalence of viruses among mice and rats of vivarium institutes in Almaty city diagnosed by using rt-PCR method

Facility	Mice		Rats	
	No. positive / Total No.	Pathogens*	No. positive / Total No.	Pathogens*
University 1	10/10	all	10/10	all
University 2	10/10	all	10/10	all
University 3	0/10	no	-	-
Institute 1	10/10	all	10/10	all
Institute 2	0/10	no	0/10	no
Manufacturer (NSCEDI)	0/10	no	0/10	no

*Mouse parvovirus NS1, Mouse parvovirus VP2, Minute virus of mice, Kilham rat virus and Rat parvovirus VP2.

Table 4: ELISA results of mice and rat blood sera from SPF animal manufacturer (NSCEDI)

Pathogen	No. positive / Total No.
Mice	
Mouse Hepatitis Virus	0/10
Mouse rotavirus (EDIM)	0/10
Theiler's murine encephalomyelitis virus	0/10
Murine norovirus	0/10
Rats	
Rat minute virus	0/10
Pneumonia virus of mice	0/10
Rat coronavirus	0/10
Rat Theilovirus	0/10

CONCLUSION

The present study provides the first published data on the prevalence of certain pathogens in colonies of laboratory animals in an institution in Almaty city. Our results show a high prevalence of FELASA-regulated pathogens among conventional laboratory animals. In contrast, the care and use of SPF animals in IVC protected animals from infection with pathogens. However, the results are limited. Longer studies are needed, involving more facilities in Kazakhstan, which keep and use laboratory animals.

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CONFLICT OF INTEREST

The authors declared no potential conflict of interest.

NOVELTY STATEMENT

The article presents for the first time the results of the spread of some infectious agents in the colonies of laboratory animals of universities and academic institutions in Almaty.

AUTHORS CONTRIBUTION

Rinat Islamov, Dinara Turegeldieva, and Altyn Rysbekova designed and wrote the article. Kuralay Sarmantayeva, and Victor Semenuyk performed research. All authors have read and approved the final manuscript.

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