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Studying dynamic changes in body mass and mass of internal organs in laboratory rats experimentally infected with bovine leukosis virus

E. S. Krasnikova[1], R. V. Radionov[2], A. V. Krasnikov[3], A. Yu. Svetozarova[4]

[1,3,4] FSBEI HE "Michurinsk State Agrarian University" (FSBEI HE Michurinsk SAU), Michurinsk, Russia

[2] FSBEU HE "Saratov State Agrarian University named after N. I. Vavilov" (FSBEU HE Saratov SAU), Saratov, Russia

[1] *ORCID 0000-0003-4395-5862, e-mail: krasnikovaes77@yandex.ru*

[2] *ORCID 0000-0002-8586-2691, e-mail: roman5875@mail.ru*

[3] *ORCID 0000-0002-4127-8725, e-mail: krasnikov.77@mail.ru*

[4] *ORCID 0000-0002-3412-527X, e-mail: ana.svetozarova@yandex.ru*

SUMMARY

Enzootic bovine leukosis has been an urgent problem of veterinary medicine both in the Russian Federation and abroad for more than a hundred years. A number of aspects have been studied quite deeply; however, there are still areas that require additional research. These include the development of a fully adequate laboratory model for reproducing bovine leukosis virus (BLV) infection. Preliminary studies have established that BLV infection of laboratory rats is accompanied by clinical, morphological and biochemical changes in the blood, signs of immune suppression, impaired immunological reactivity of the body, and morphofunctional changes in the immunocompetent cells that correlate with bovine leukosis. In this regard, it is of interest to analyze disorders caused by these dysfunctions; the disorders are demonstrated by changed morphometric characteristics of both the body and individual organs. The aim of the research was to study dynamic changes in body mass and mass of internal organs in laboratory rats experimentally infected with BLV. There was a clear body mass increase in BLV-infected laboratory rats, then followed by a decrease down to negative numbers. The reverse trend was observed for such internal organs of the experimental animals as liver, spleen, kidneys and lungs. At first, their relative mass decreased to some extent, then increased with different dynamics in groups. The heart was the exception, as its relative mass decreased and did not increase until the end of the experiment. The data obtained correlate with those provided by a number of authors that the relative mass of various organs changes in the BLV infected animals because of proliferative, inflammatory, dystrophic and atrophic processes.

Keywords: Rats, enzootic bovine leukosis, relative mass, internal organs, average daily mass gain, relative mass gain.

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For correspondence: Ekaterina S. Krasnikova, Doctor of Science (Veterinary Medicine), Associate Professor, Professor, Department of Animal Science and Veterinary Medicine, FSBEI HE Michurinsk SAU, 393760, Russia, Tambov Oblast, Michurinsk, Internatsyonalnaya str., 101, *e-mail: krasnikovaes77@yandex.ru.*

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Изучение динамики массы тела и внутренних органов лабораторных крыс при экспериментальной инфекции вирусом лейкоза крупного рогатого скота

Е. С. Красникова[1], Р. В. Радионов[2], А. В. Красников[3], А. Ю. Светозарова[4]

[1,3,4] ФГБОУ ВО «Мичуринский государственный аграрный университет» (ФГБОУ ВО Мичуринский ГАУ), г. Мичуринск, Россия

[2] ФГБОУ ВО «Саратовский государственный аграрный университет имени Н. И. Вавилова» (ФГБОУ ВО Саратовский ГАУ), г. Саратов, Россия

[1] *ORCID 0000-0003-4395-5862, e-mail: krasnikovaes77@yandex.ru*

[2] *ORCID 0000-0002-8586-2691, e-mail: roman5875@mail.ru*

[3] *ORCID 0000-0002-4127-8725, e-mail: krasnikov.77@mail.ru*

[4] *ORCID 0000-0002-3412-527X, e-mail: ana.svetozarova@yandex.ru*

РЕЗЮМЕ

Энзоотический лейкоз крупного рогатого скота уже более ста лет является актуальной проблемой ветеринарной медицины как в Российской Федерации, так и за рубежом. Ряд аспектов изучен достаточно глубоко, но есть и такие, что требуют проведения дополнительных исследований. К их числу относится разработка адекватной во всех отношениях лабораторной модели для воспроизведения инфекции, вызванной вирусом лейкоза крупного рогатого скота (BLV-инфекция). Предварительными исследованиями было установлено, что BLV-инфекция лабораторных крыс сопровождается коррелирующими с лейкозом у крупного рогатого скота клинико-морфологическими и биохимическими изменениями в крови, признаками иммунной супрессии, нарушением иммунологической реактивности организма и морфофункциональными изменениями на уровне иммунокомпетентных клеток. В этой связи интерес представляет анализ провоцируемых данными дисфункциями нарушений, находящих свое отражение в изменении морфометрических характеристик как всего организма, так и отдельных органов. Целью исследований стало изучение динамики массы тела и внутренних органов лабораторных крыс при экспериментальной BLV-инфекции. Динамика весовых показателей тела BLV-инфицированных лабораторных крыс характеризовалась выраженной тенденцией к их увеличению с последующим снижением вплоть до отрицательных значений. Обратная тенденция была отмечена для таких внутренних органов экспериментальных животных, как печень, селезенка, почки и легкие. Сначала их относительная масса в той или иной степени снижалась, затем увеличивалась с разной динамикой по группам. Исключение составило сердце, относительная масса которого снизилась и не увеличивалась до окончания эксперимента. Полученные данные коррелируют с мнением ряда авторов, что при BLV-инфекции относительная масса различных органов изменяется в результате пролиферативных, воспалительных, дистрофических и атрофических процессов.

Ключевые слова: Крысы, энзоотический лейкоз крупного рогатого скота, относительная масса, внутренние органы, среднесуточный привес, относительный привес.

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Для корреспонденции: Красникова Екатерина Сергеевна, доктор ветеринарных наук, доцент, профессор кафедры зоотехнии и ветеринарии ФГБОУ ВО Мичуринский ГАУ, 393760, Россия, Тамбовская обл., г. Мичуринск, ул. Интернациональная, д. 101, *e-mail: krasnikovaes77@yandex.ru.*

INTRODUCTION

Enzootic bovine leukosis (EBL) – a widespread disease, especially often detected in highly productive dairy cows [1]. Cattle, sheep, and rabbits are used as models to study pathological processes in the experimentally infected animals [2]. In the previous studies, Wistar laboratory rats demonstrated susceptibility to oral and parenteral infection with EBL [3, 4]. Since it was found that laboratory animals infected with bovine leukosis virus (BLV) demonstrate clinical, morphological and biochemical changes in blood that correlate with enzootic bovine leukosis [3], signs of immune suppression and impaired immunological reactivity of the body [4], as well as morphofunctional changes at the level of immunocompetent cells [5], Wistar rats can be considered as a new laboratory model for studying BLV *in vivo*. Such conclusions require a correlation (at the level of disease pathogenesis) between laboratory and naturally susceptible animals. Therefore, it is of particular interest to analyze the disorders caused by these dysfunctions, i.e. those disorders that are demonstrated by changed morphometric characteristics of both the body and individual organs.

In this regard, the aim of the research was to study dynamic changes in body mass and mass of internal organs of laboratory rats experimentally infected with BLV.

MATERIALS AND METHODS

Wistar rats (n = 60) divided into three equal groups were used as an object of the study. The rats had an adequate diet and daily received plenty of fresh milk from the BLV-infected and diseased cows (as reported by the state veterinary service), originating from collective farm "Zarya" located in the Tamalinsky District of the Penza Oblast. Rats of Group One (I) were fed on milk from intact cows, Group Two (II) was fed on milk from BLV-infected cows and Group Three (III) was fed on milk from the cows with clinical form of bovine leukosis. The animals of each group were divided into 2 subgroups: *a* – included adult rats, *b* – included rat pups. The rat pups born during the experiment were separated from their mothers after they started self-feeding. Polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) were used to follow the dynamics of BLV infection in experimental rats 3, 6, 9 and 12 months after the experiment started.

Blood was aspirated from the lateral caudal vein into vacuum tubes with aEDTA-K3 stabilizer (ethylenediaminetetraacetic acid) and into test tubes with clot activator (PUTH, China). For PCR diagnostics the following reagent kits were used: "DNA-sorb-B", "LEUKEMIA" and "EF" (Federal State Budgetary Institution of the Central Research Institute of Epidemiology of the Rospotrebnadzor,

Table
Dynamics of BLV-infection in rats of the experimental group

Таблица
Динамика развития BLV-инфекции у крыс экспериментальных групп

Test dates	Groups and subgroups of animals											
	I*a*		I*b*		II*a*		II*b*		III*a*		III*b*	
	PCR	ELISA	PCR	ELISA	PCR	ELISA	PCR	ELISA	PCR	ELISA	PCR	ELISA
3 months after	–	–	–	–	+	+	–	+	+	–	X	X
6 months after	–	–	–	–	+	+	+	+	+	+	+	+
9 months after	–	–	–	–	+	+	+	+	+	+	+	+
12 months after	–	–	–	–	+	+	+	+	+	+	+	+

"–" – negative result (отрицательный результат);
"+" – positive result (положительный результат);
"X" – not tested (исследования не проводились).

Russia); the PCR procedure was held and the tests result were read in Bio-Rad Laboratories equipment, Inc. (USA). Serological tests were performed using a "BLV antibody ELISA kit for serum and milk (option No. 1 – screening)" produced by the FKP "Kursk Biofactory – Firm BIOK" (Russia) using Multiskan equipment (Thermo Scientific, USA). To confirm BLV infection in experimental rats, presence ("+") or absence ("–") of proviral DNA and/or antibodies were taken into account as qualitative indicators. Animals that were positive for at least one of the indicators were used in the experiment, and negatively reacting rats were culled.

Five rats from each group were subjected to euthanasia and autopsy within the established time limits. The animals were euthanized by cervical dislocation after diethyl ether anesthesia.

All the experiments on animals were carried out strictly in accordance with the Interstate guidelines for accommodation and care of laboratory animals GOST 33216-2014 and GOST 33215-2014, adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22.09.2010 on the protection of animals used for scientific purposes.

Parenchymal organs of animals: kidneys, liver, spleen, heart and lungs were used as materials for morphometric studies. Electronic scales JW-1 (*e* = 0.02 g) manufactured by Acom Inc. (South Korea) were used to weigh rats and their internal organs.

RESULTS AND DISCUSSION

The results of the serological, molecular, and genetic tests presented in the table indicate positive dynamics of the infectious process in experimental animals, i.e. at least one of the diagnostic tests gave a positive result for the group. This was most likely due to biological characteristics of the infection causative agent and by the peculiarities of the disease pathogenesis. The animals of the control group remained intact throughout the whole experiment.

The data on the body mass of experimental animals indicates that the positive trend reported at the beginning of

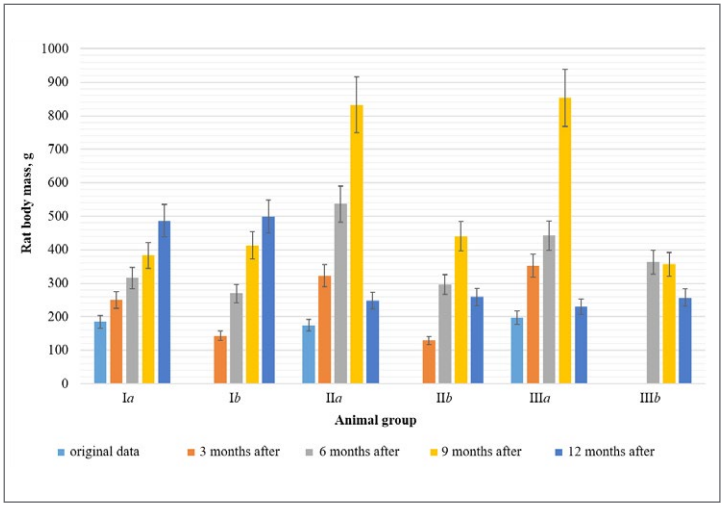

Fig. 1. Dynamic changes in animal body mass

Рис. 1. Динамика изменения массы тела животных

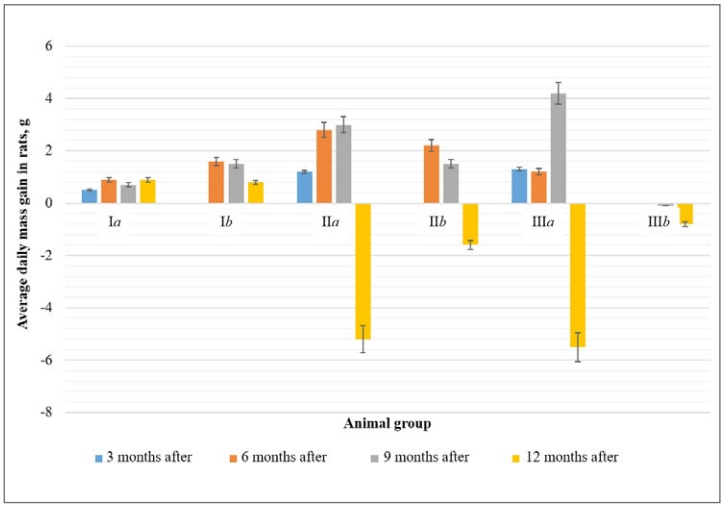

Fig. 2. Dynamic changes in average daily mass gain

Рис. 2. Динамика среднесуточного прироста массы тела

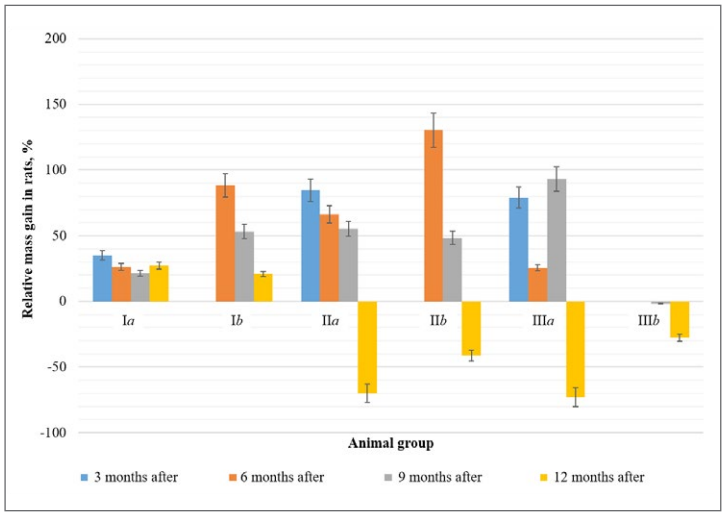

Fig. 3. Dynamics of relative body mass gain in animals

Рис. 3. Динамика относительного прироста массы тела животных

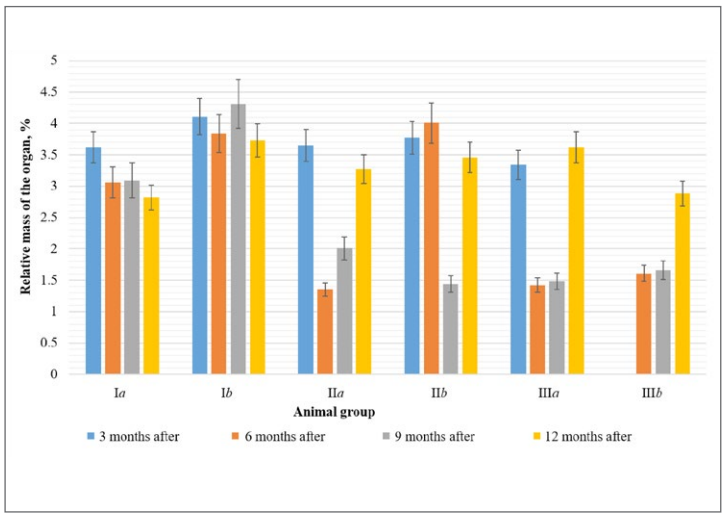

Fig. 4. Dynamic changes in relative mass of animal liver

Рис. 4. Динамика относительной массы печени животных

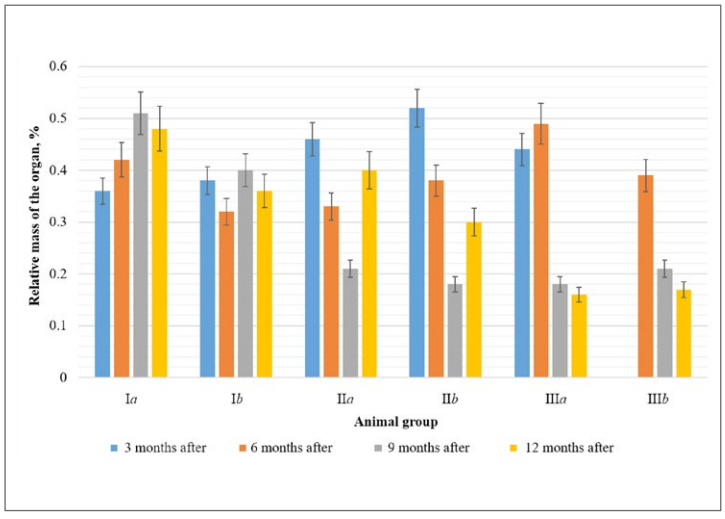

Fig. 5. Dynamic changes in relative mass of animal spleen

Рис. 5. Динамика относительной массы селезенки животных

the experiment was replaced by a negative one at the end of the observation period, and cachexia began to develop in the experimental animals following a sharp increase in the body mass. This was most pronounced in the rat pups of Group III, where absolutely no positive dynamics were reported. At the same time, the control group rats naturally gained weight. The changes in the body mass of animals and the dynamic changes in the average daily and relative mass gain of rats are shown in Figures 1–3.

As Figure 1 shows, the animals in the experimental groups had pronounced positive dynamics in the body mass change during 9 months of the experiment and sharp negative dynamics at the end of the experiment. By the third quarter of the experiment, the body mass of adult rats in the experimental groups was 2.2 times more than that in the control group, and the body mass of their pups was 1.5 times more compared to the control. The obesity in rats may be related to an increased fat content of milk from the BLV-diseased and infected cows, which is often caused by a decrease in milk volume resulting from subclinical mastitis [6]. By the end of the experiment, the body mass of rats in Groups II*a* and III*a* decreased by 3.4 and 3.7 times in comparison to the previous indicators, and the body mass of their pups decreased by 1.7 and 1.4 times, respectively, whereas the body mass of the intact animals continued to increase gradually. The steady development of cachexia may indicate changes in the metabolism of the experimental animals, since there is evidence that chronic viral infections induce metabolic disorders [7], and bovine leukosis is often accompanied by cachexia [8].

Figure 2 shows that the most pronounced positive dynamic changes in the average daily mass gain was observed in the first 9 months of the experiment in adult rats that had milk from BLV-diseased and infected cows, then the indicator became sharply negative in these animals. At the beginning of the experiment, the rat pups from Group II rats showed an increase in body mass, but by the end of the research, this indicator became negative, as well as in the parent population. However, the average daily mass gain of the rat pups from Group III first remained at zero and then slightly decreased. Dynamic changes in the average daily mass gain in animals directly correlates with the indicators of the relative mass gain, and the corresponding trends are shown in Figure 3.

The dynamic changes in the relative mass gain of rats in all groups had a negative trend (Fig. 3). The exception was observed in adult rats, whose diet included milk from cows with bovine leukosis, relative mass gain indicators in animals of this group were not constant. By the first quarter of the experiment, this indicator in Groups II*a* and III*a* was (84.6 ± 7.8)% and (78.9 ± 7.1)%, respectively, exceeding that in animals of the control group by 2.4 and 2.3 times. However, 6 and 9 months after the start of the experiment, the relative mass gain in Group II*a* animals decreased by 1.3 and 1.2 times, respectively, whereas it first sharply decreased by 3.1 times in Group III*a* rats and then increased by 3.6 times, which can be associated with metabolic changes most likely caused by the hormonal background of the animals. By the end of the experiment, this indicator was negative in all experimental groups and ranged from –(27.8 ± 2.1)% and –(41.1 ± 3.8)% in the pups from Groups III and II to –(73.0 ± 7.1)% and –(70.2 ± 6.6)% in the parent population, respectively; however, this indicator was positive in the control animals and remained at (20.9 ± 1.7)% and (27.1 ± 2.1)%.

Relative mass of the internal organs is an important criterion that characterizes the state of the body. It is known that the mass of any organ is directly related to its functional state. At the same time, changes of the organ volume and structure can be caused either by the age-related changes, or by any pathological processes [9]. Dynamic changes in the relative mass of the internal organs of the experimental animals demonstrated certain trends in different groups. The data obtained are illustrated in Figures 4–8.

Relative liver mass of the experimental rats initially showed a negative trend (Fig. 4), most likely due to a rapid increase in the body mass. By the end of the experiment, this indicator significantly increased in all experimental groups, which may be caused by cachexia, as well as by inflammatory processes and intoxication, which markers were identified during clinical, morphological and biochemical tests of blood from experimental animals [10, 11]. So, 3 and 6 months after the start of the experiment, the relative liver mass of rats of Groups II*a* and III*a* reduced by 2.3/2.2 and 1.5/2.1 times, respectively, in comparison to the control group and by the end of the experiment, this indicator already exceeded the indicators of the control group by 1.2 and 1.3 times. In the middle of the experiment relative liver mass of experiment rat pups was lower by 3.0 and 2.6 times compared to the control, and at the end – this indicator increased by 2.4 and 1.7 times compared to the previous data; whereas the control rat pups demonstrated approximately the same numbers over the entire observation period.

Relative spleen mass of animals changed during the experiment in the following way (Fig. 5). It first decreased in experimental rats by 1.4–2.1 times, varying from group to group, in connection with the body mass gain and with the exception of Group III adult animals, where the relative spleen mass first slightly increased by 1.1 times, and then sharply decreased by 2.7 times. Then, this indicator changed in Groups II*a* and II*b* and the organ increased by 1.9 and 1.6 times at the end of the experiment, which was, probably, caused either by inflammatory or proliferative processes in the spleen alongside with a decrease in the body mass gain. Group III rats and their pups demonstrated a progressive reduction in the organ volume by 3.0 and 2.1 times, respectively, compared with the control. The trend was possibly associated with atrophic processes.

Six-nine month after the start of the experiment, relative kidney mass of rats from the experimental groups (Fig. 6) decreased by 1.6/1.8/2.5 compared to the initial data for Groups II*a*/II*b*/III*a*, alongside with the body mass increase. At the end of the experiment, there was an increase in the relative mass of the organ in all the experimental groups by 1.2–1.9 times as compared to the data obtained in the third quarter. The increase may be associated with proliferative processes or kidneys hypertrophy due to intoxication. Group III*b* rats were an exception with a constant increase in the relative kidney mass, because there was not an initial increase in the relative body mass gain. It should be noted that the relative kidney mass of the experimental rat pups was 1.2 times higher than that of the rat pups from the intact animals. In adult rats from Group II this indicator was 1.3 times higher, and in rats from Group III it was 1.6 times lower than that in the intact animals.

As Figure 7 shows, relative mass of the rat lungs did not change so dynamically as in case of other organs

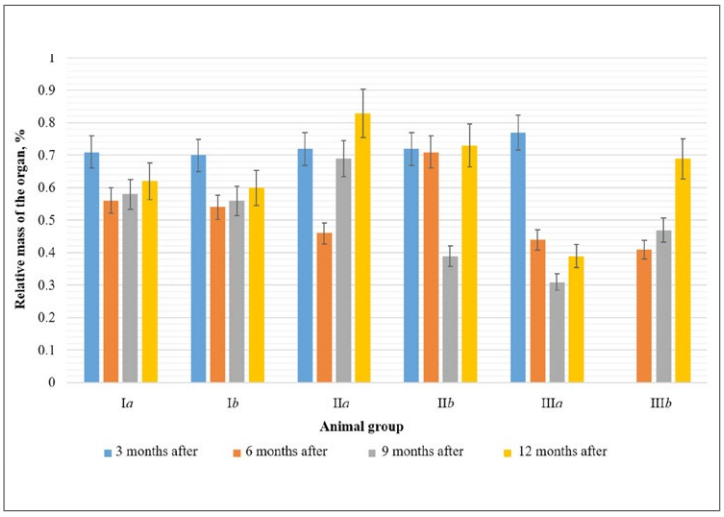

Fig. 6. Dynamic changes in relative mass of animal kidneys

Рис. 6. Динамика относительной массы почек животных

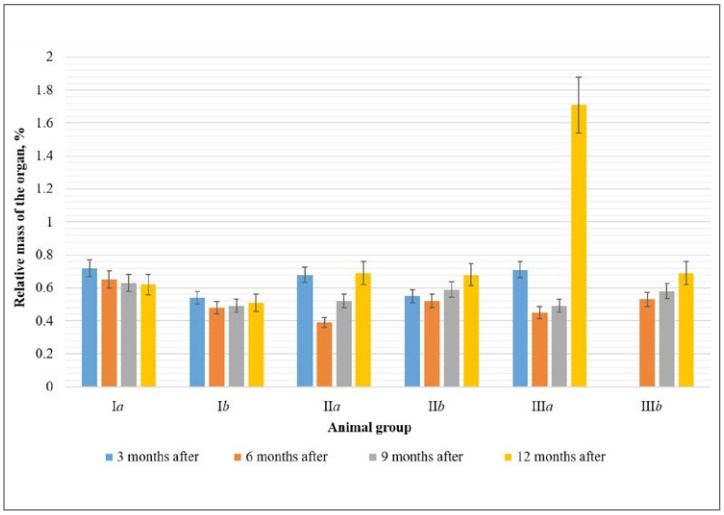

Fig. 7. Dynamic changes in relative mass of animal lungs

Рис. 7. Динамика относительной массы легких животных

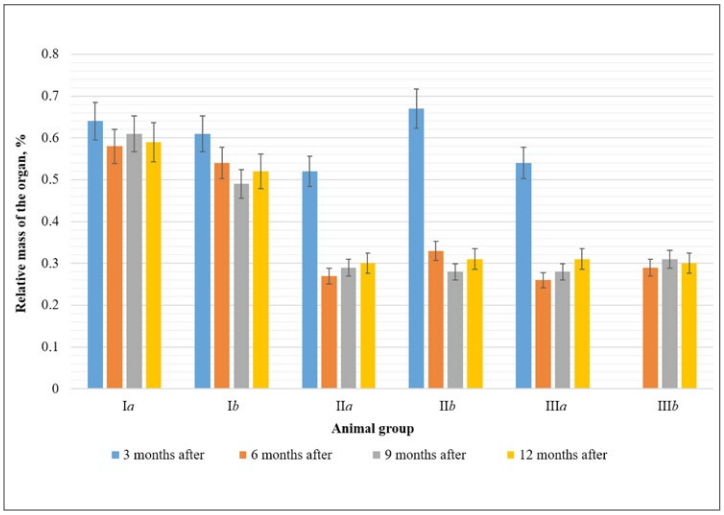

Fig. 8. Dynamic changes in relative mass of animal heart

Рис. 8. Динамика относительной массы сердца животных

described above. It increased sharply only in animals from Group III*a*. At the end of the experiment, the indicator in the given group exceeded that one in the control group by 2.8 times. This indicator increased by 1.3 times in the pups from Groups II and III, as compared to the intact ones. This was due to autopsy-confirmed single and double pneumonia in many experimental rats which could be triggered by BLV-induced immunosuppression. According to N. G. Kozyreva et al. [2], P. Dimitrov et al. [12], pneumonia often accompanies experimental BLV infection in rabbits, which confirms the research results.

Relative heart mass in animals from all the experimental groups demonstrated a dynamic decrease by 2.5–3.0 times compared to the control (Fig. 8), despite cachexia reported at the end of the experiment. This may be due to the progressive atrophy or dystrophy of the organ alongside with metabolic disorders.

CONCLUSION

Thus, the obtained results demonstrate that the experimental BLV infection is accompanied by regular changes in both absolute and relative indicators of body mass and mass of internal organs in Wistar laboratory rats. There was a clear body mass increase in BLV-infected laboratory rats, then followed by a decrease down to negative numbers. The reverse trend was observed for such internal organs of the experimental animals as liver, spleen, kidneys and lungs. At the beginning of the experiment, their relative mass decreased to some extent, then increased with different dynamics in groups. The heart was the exception, as its relative mass decreased and did not increase until the end of the experiment.

The data obtained correlate with the results provided by other authors stating that BLV infection induces disorders not only in the hematopoietic, but also in other vital organs of the animal. As a result of proliferative, inflammatory, dystrophic and atrophic processes, the relative mass of various organs changes [13], with the most pronounced changes found in the spleen, liver, kidneys and heart [14–16].

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Ekaterina S. Krasnikova, Doctor of Science (Veterinary Medicine), Associate Professor, Professor, Department of Animal Science and Veterinary Medicine, FSBEI HE Michurinsk SAU, Michurinsk, Russia.

Roman V. Radionov, Candidate of Science (Biology), Assistant, Department of Animal Diseases and Veterinary and Sanitary Expertise, FSBEU HE Saratov SAU, Saratov, Russia.

Aleksander V. Krasnikov, Doctor of Science (Veterinary Medicine), Associate Professor, Head of the Department of Animal Science and Veterinary Medicine, FSBEI HE Michurinsk SAU, Michurinsk, Russia.

Anna Yu. Svetozarova, Post-Graduate Student, Department of Animal Science and Veterinary Medicine, FSBEI HE Michurinsk SAU, Michurinsk, Russia.

Красникова Екатерина Сергеевна, доктор ветеринарных наук, доцент, профессор кафедры зоотехнии и ветеринарии ФГБОУ ВО Мичуринский ГАУ, г. Мичуринск, Россия.

Радионов Роман Владимирович, кандидат биологических наук, ассистент кафедры болезней животных и ветеринарно-санитарной экспертизы ФГБОУ ВО Саратовский ГАУ, г. Саратов, Россия.

Красников Александр Владимирович, доктор ветеринарных наук, доцент, заведующий кафедрой зоотехнии и ветеринарии ФГБОУ ВО Мичуринский ГАУ, г. Мичуринск, Россия.

Светозарова Анна Юрьевна, аспирант кафедры зоотехнии и ветеринарии ФГБОУ ВО Мичуринский ГАУ, г. Мичуринск, Россия.