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A COMPARATIVE STUDY OF *OPISTRORCHIS FELINEUS* (RIVOLTA, 1884) INFECTION IN INBRED C57BL/6 AND OUTBRED CD-1 MICE

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In the present work, we conducted a comparative study on inbred C57BL/6 and outbred CD-1 mice after injection of *O. felineus* larvae. Body weight and weights of the liver and spleen as well as biochemical parameters of blood serum were evaluated at 4 and 6 weeks postinfection. An increased relative weight of the liver was observed after 4 weeks in outbred CD-1 mice and after 6 weeks in inbred C57BL/6 mice. In response to the infection, in CD-1 mice after 4 weeks, the relative weight of the spleen increased and returned to baseline 6 weeks postinfection. Alanine aminotransferase activity in C57BL/6 mice, compared to CD-1 mice, was substantially higher than the control value 6 weeks after the injection of *O. felineus* larvae. In CD-1 mice, the *O. felineus* infection contributed to a significant increase in the activity of aspartate aminotransferase and to a modest upregulation of γ-glutamyl transpeptidase after 4 weeks. Both of these parameters normalized 6 weeks postinfection. Furthermore, inbred C57BL/6 mice had significantly more maritae of *O. felineus* in hepatic biliary ducts than outbred CD-1 mice did. Meanwhile, in CD-1 mice, the number of maritae decreased by more than twofold at 6 weeks postinfection as compared to 4 weeks postinfection. Our results revealed differences in susceptibility to *O. felineus* infection between the mouse strains (depending on the genotype) and indicate that inbred C57BL/6 mice are preferable as a model for studies on experimental *O. felineus*-induced opisthorchiasis.

Key words: *O. felineus*, mice C57BL/6 and CD-1, differing susceptibility.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ *OPISTRORCHIS FELINEUS* (RIVOLTA, 1884) ИНФЕКЦИИ У МЫШЕЙ ИНБРЕДНОЙ ЛИНИИ C57BL/6 И АУТБРЕДНОЙ CD-1

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В настоящей работе было проведено сравнительное исследование инбредных мышей C57BL/6 и аутбредных CD-1 после введения им личинок *O. felineus*. Оценивали массу тела, печени и селезенки, а также биохимические показатели в сыворотке крови через 4 и 6 недель после инфицирования животных. Увеличенная относительная масса печени была зафиксирована через 4 недели у аутбредных мышей CD-1 и через 6 недель у инбредных мышей C57BL/6. В ответ на инфицирование у мышей CD-1 через 4 недели увеличивалась относительная масса селезенки, которая возвращалась к норме через 6 недель. Активность аланинаминотрансферазы у мышей C57BL/6 по сравнению с мышами CD-1 через 6 недель после введения личинок *O. felineus* была существенно выше контрольного значения. У мышей CD-1 инфицирование *O. felineus* способствовало статистически значимому повышению активности аспартатаминотрансферазы и некоторому повышению гамма-глутамилтрансферазы через 4 недели, которые нормализовались через 6 недель. При этом инбредные мыши C57BL/6 содержали в желчных протоках печени значительно больше марит *O. felineus*, чем аутбредные мыши CD-1. Причем у мышей CD-1 через 6 недель после инфицирования число марит уменьшилось более чем в 2 раза по сравнению со сроком 4 недели. Полученные данные, выявляющие разную восприимчивость животных к инфицированию *O. felineus* в зависимости от генотипа, предполагают предпочтительное использование инбредных мышей C57BL/6 в качестве модельных при исследовании экспериментального *O. felineus*-индуцированного описторхоза.

Ключевые слова: *O. felineus*, мыши C57BL/6 и CD-1, разная чувствительность.

Opisthorchiasis is a severe parasitosis of humans and animals which induced by invasion of larvae of *Opisthorchis felineus* (Rivolta, 1884) or *O. viverrini* (Poirier, 1886) (fam. Opisthorchiidae). This infection takes place after consumption of raw or undercooked fish from the fam. Cyprinidae. The endemic area of *O. viverrini* is Southeast Asia, especially Northeast Thailand (Sripa, Kaewkes, 2002; Fürst et al., 2012; Sripa et al., 2017). The species *O. felineus* is widespread in Central and Western Eurasia (Keiser, Utzinger, 2005) covering nine countries (Fürst et al., 2012). The peak of prevalence of *O. felineus*-induced opisthorchiasis in Western Siberia is observed in the Ob-Irtysh region (Mordvinov, Furman, 2010; Ogorodova et al., 2015). Overall, 35 million people worldwide are affected by Opisthorchiidae-related diseases (Ogorodova et al., 2015). Opisthorchiasis is dangerous not only because it causes pathologies of the hepatobiliary system (purulent cholangitis, chronic hepatitis, stenosis of hepatobiliary

ducts, and abscesses of the liver) but also because it enhances or triggers diseases of other organs and systems of organism, for example, in the pancreas, in organs of the gastrointestinal tract, broncho-pulmonary system, and the thyroid gland (Bronstein, Luchshev, 1998; Sripa, 2003; Akhmedov, Kritevich, 2009). For these reasons, modeling of opisthorchiasis in laboratory experiments and research into the pathogenesis are highly relevant topics at present.

For modeling of opisthorchiasis, researchers often use golden hamsters *Mesocricetus auratus* (Sripa, Kaewkes, 2002; Stepanova, Podkletnova, 2002; Sripa, 2003; Boonmars et al., 2009). There are studies where — for infection with *O. felineus* or *O. viverrini* — researchers used silver foxes *Vulpes vulpes fulva* Desmarest, 1820 (Schuster et al., 2003), guinea pigs and kittens (Glumov et al., 1986), rodents (fam. Muridae): the jirds *Meriones unguiculatus* (Milne-Edwards, 1867) (Adam et al., 1993), and mice (Nair et al., 2011). Nonetheless, some investigators believe that mice and rats are not optimal model animals for studies on opisthorchiasis processes as opposed to hamsters and voles (Boonmars et al., 2009).

On the other hand, there is little doubt that mice are preferable laboratory animals for virtually any biomedical studies. Inbred strains of mice such as C57BL/6, BALB/c, SCID, and A/J and outbred CD-1 mice are most frequently used as physiological or pathological models for experiments *in vivo* (Labome, 2012). In parasitological studies, it was shown that the C57BL/6 strain of mice is the most susceptible to hepatic infection with *Plasmodium berghei* (Vincke et Lips, 1948) sporozoites (among the six analyzed strains of laboratory mice) (Scheller et al., 1994) and to infection with liver fluke *Fasciola hepatica* Linnaeus, 1758 (among the 10 tested inbred strains of mice) (Andrews, Meister, 1978).

It should be noted that inbreeding is associated with profound effects on the immune system by reducing immunocompetence and resistance to pathogens and parasites, as suggested by some authors (Ilmonen et al., 2008; Hofer et al., 2010). At the same time, outbred mice are closer to natural mammalian populations, including human ones (Hofer et al., 2010). Therefore, those authors believe that the use of outbred mice should be thoroughly studied, instead of the popular inbred strains, for example, in experiments evaluating the effects of pharmacological interventions on hematopoiesis. In contradiction to this point of view, in other studies, it was shown that various inbred strains of mice can differ severalfold in susceptibility toward some parasitic species, e. g., one of the strains of *Leishmania mexicana* Biagi, 1953, whereas outbred CD-1 mice occupy an intermediary position in this regard (Neal, Hale, 1983). Accordingly, it was important to determine how infection with *O. felineus* affects mice with varying degrees of homozygosity of inherited traits: inbred and outbred animals. In the early studies, C57BL/6J mice were regarded as the least susceptible to *O. felineus* infection because 2 months after injection of metacercariae, their liver does not contain a single pubescent fluke, but immature maritae and dead worms are present (Zelentsov, 1974). In contrast, in our previous studies, it was demonstrated that C57BL/6 mice are sufficiently susceptible to infection with *O. felineus*, and 2 weeks postinfection, hepatic bile ducts of these mice contain the same number of maritae as in golden hamsters (Avgustinovich et al., 2017). Susceptibility of outbred CD-1 mice to infection with *O. felineus* has not been studied to date.

Therefore, the aim of this study was to create experimental models of *O. felineus*-induced opisthorchiasis in inbred C57BL/6 and outbred CD-1 mice. Consequences of the infection were assessed, first of all, by the number and maturity of maritae in the hepatobiliary system, by the relative weight of the liver and spleen, and by changes in blood serum activities of the enzymes that reflect the extent of damage to liver cells: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGT). Analysis of these parameters was conducted 4 and 6 weeks postinfection.

MATERIAL AND METHODS

We used male mice (weighing 26—36 g, age 3—4 months) of inbred strain C57BL/6 and outbred strain CD-1. The animals were obtained from the Center for Genetic Resources of Laboratory Animals at the Federal Research Center Institute of Cytology and Genetics, SB RAS (RFMEFI61914X0005 and RFMEFI61914X0010). The mice were maintained in cages 36 × 23 × 12 cm (2—7 individuals per cage) under the light regimen 12 h/12 h (light/darkness) at air temperature 23—24 °C. Granulated feed and water were available *ad libitum*. The handling and care of mice were conducted in strict accordance with the recommendations of the European Communities Council Directive of 24 November 1986 (86/609/EEC). The study protocol was approved by the Committee on the Ethics of Animal Experiments of the Institute of Cytology and Genetics, SB RAS (protocol N 22 of May 30, 2014).

Pubescent male mice of inbred strain C57BL/6 and outbred CD-1 strains were distributed in a random manner into two series by duration of *O. felineus* infection: 4 and 6 weeks. Each series contained a control group of mice, which received a one-time injection of physiological saline, and an experimental group, with injection of 100 metacercariae per mouse. These were intragastric injections by means of a probe (Braintree Scientific, Inc.): 100—150 μL of the suspension per 10 g of body weight of a mouse. Four and 6 weeks after the infection, the mice were euthanized by rapid decapitation, after which, blood was collected for biochemical analysis. Whole blood from each mouse, after staying under ambient conditions for 2—3 h to achieve good clotting, was then centrifuged for 25 min (4 °C and 1000 × g), and serum was separated and stored at –70 °C until biochemical analysis. Besides, relative weights of the liver and spleen were determined (per gram of body weight of a mouse). The liver and gall bladder were placed in physiological saline for subsequent microscopic examination of the number of adult *O. felineus* individuals in bile ducts and for analysis of their maturity.

Larvae of *O. felineus* were obtained from infected ides (*Leuciscus idus*) caught in the Ob river of Novosibirsk region. Ground meat of the fish was digested with a 1% solution of pepsin chloride (incubation at 37 °C overnight), followed by filtration and precipitation in 0.9 % NaCl. A light microscope was used to determine viability of the metacercariae, which were then used for injections into mice.

The mouse liver was examined under a light microscope (MBS-10, × 16) for the presence of *O. felineus* in bile ducts and in the gall bladder. If maritae were detected, then using two preparation needles, we squeezed out the duct

contents and transferred them by means of a micropipette into a Petri dish containing physiological saline. After bile clots were washed away, the worms were counted and placed on a histological slide for photography. The imaging was conducted at the Multiple-Access Center for Microscopy of Biological Subjects (Institute of Cytology and Genetics, Novosibirsk, Russia) (http://www.bionet.nsc.ru/microscopy/) under an Axioskop 2 Plus microscope, equipped with an AnxioCamp HRc camera (Zeiss, Germany).

The collected serum was assayed using a Dimension RxL Max automatic clinical chemistry system (Dade Behring Inc., USA). ALT, AST, and GGT activities were determined using commercially available cartridges according to the manufacturer's instructions (Dimension Clinical Kit, Siemens, USA).

Statistical analysis of the data was performed with Statistica 6.0 software. Differences in the number of maritae in bile ducts of the mice of the two strains were evaluated by the *t*-test for independent groups of animals. Differences in body weight, liver and spleen weights, and in biochemical parameters were evaluated by a three-way ANOVA, followed by Fisher's LSD test. The first factor was the strain of mice (C57BL/6 or CD-1), the second was duration of infection (4 or 6 weeks), and the third factor was the group of mice (control or experimental). The data are presented as mean ± SEM. The groups contained 9—15 animals. Differences were considered statistically significant at $p \leq 0.05$ and were regarded as an insignificant trend at $0.05 < p < 0.1$.

RESULTS

As shown in table 1, after 4 and 6 weeks since *O. felineus* metacercariae were injected, the number of maritae in the bile ducts differed substantially between the two strains of mice: significantly more in C57BL/6 mice than in CD-1 mice after 4 weeks ($t = 2.85$; $p = 0.010$) and after 6 weeks ($t = 2.50$; $p = 0.019$). Meanwhile, in CD-1 mice at 6 weeks postinfection, the number of maritae decreased more than twofold in comparison with the time point 4 weeks ($t = 2.18$; $p = 0.039$).

Both in C57BL/6 and CD-1 mice, the maritae of *O. felineus* that were isolated from hepatic bile ducts and from the gall bladder, had a length not exceeding 3 mm and looked immature (fig. 1). They contained well-pronounced filled-out branches of the intestine only.

Table 1

The number of maritae in the liver of mice of the two strains at different time points postinfection with *O. felineus*

Strain	Duration	
	4 weeks	6 weeks
C57BL/6	18.6 ± 5.6 (n = 11)	10.9 ± 4.0 (n = 12)
CD-1	3.3 ± 0.6$^{\#\#}$ (n = 12)	1.5 ± 0.5*$^{\#}$ (n = 14)

Note. * — $p < 0.05$ — compared to 4 weeks postinfection; $^{\#}$ — $p < 0.05$ and $^{\#\#}$ — $p < 0.01$ compared to C57BL/6 mice.

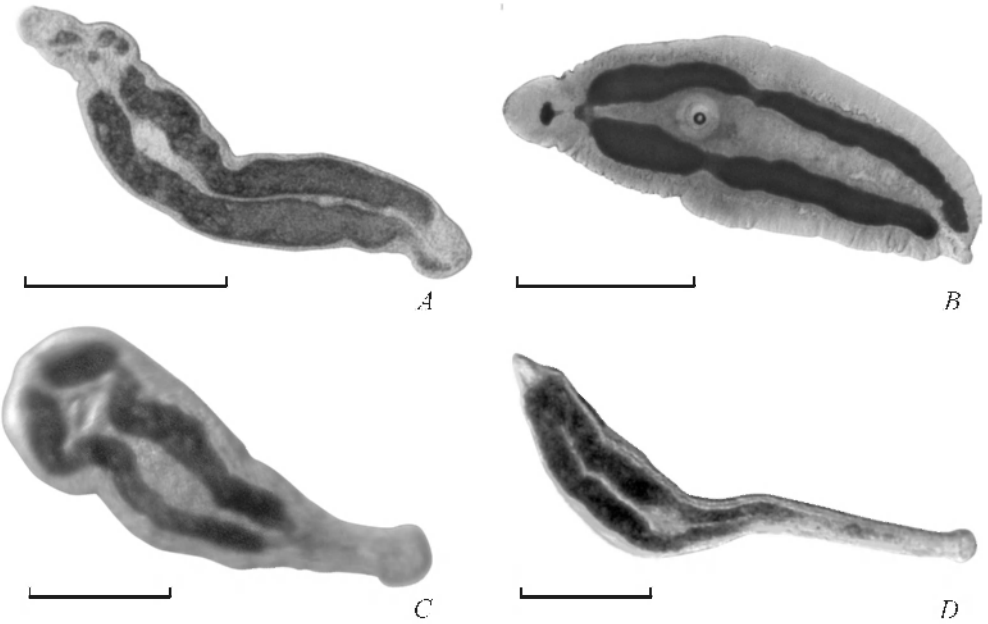

Fig. 1. *O. felineus* maritae that were isolated from the hepatic bile ducts and from the gall bladder of C57BL/6 and CD-1 mice 4 and 6 weeks postinfection (lifetime images).

A — C57BL/6 mice, 4 weeks postinfection. *B* — C57BL/6 mice, 6 weeks postinfection. *C* — CD-1 mice, 4 weeks postinfection. *D* — CD-1 nice, 6 weeks postinfection. Scale bars = 1 mm.

The mice of the two strains differed in body weight: CD-1 mice were substantially larger than C57BL/6 mice at both time points (fig. 2; table 2). Furthermore, both after 4 and after 6 weeks, the *O. felineus* infection did not influence the changes in body weight in both strains of mice (table 2).

Nonetheless, during the infection, the relative weight of the liver did change: in CD-1 mice, this alteration was observed after 4 weeks, whereas in C57BL/6 mice after 6 weeks (fig. 2; table 2). Meanwhile, after 4 weeks in the infected mice, the liver was bigger in CD-1 mice than in C57BL/6 mice, but this difference was not significant 6 weeks postinfection. There was a reduced liver weight in control C57BL/6 mice compared to control CD-1 mice after 6 weeks.

A bigger difference between the strains was observed in the relative weight of the spleen: this parameter changed in different ways in these strains in response to the infection (fig. 2; table 2). In CD-1 mice, the spleen weight was significantly increased after 4 weeks but returned to baseline 6 weeks postinfection. The two strains of the infected animals differed significantly in this parameter after 4 weeks. In C57BL/6 mice, we did not detect any notable alterations of the spleen weight in response to the infection during the study period. In the meantime, control mice of this strain had a lowered spleen weight in comparison with control CD-1 mice after 6 weeks.

As shown in table 2, there was a substantial interaction among all three factors under study during analysis of the relative spleen weight.

In the biochemical analysis, in response to infection with *O. felineus*, there were substantial alterations in enzymatic activity of ALT (fig. 3; table 3). In C57BL/6 mice, 6 weeks after injection of *O. felineus* larvae, the activity of ALT

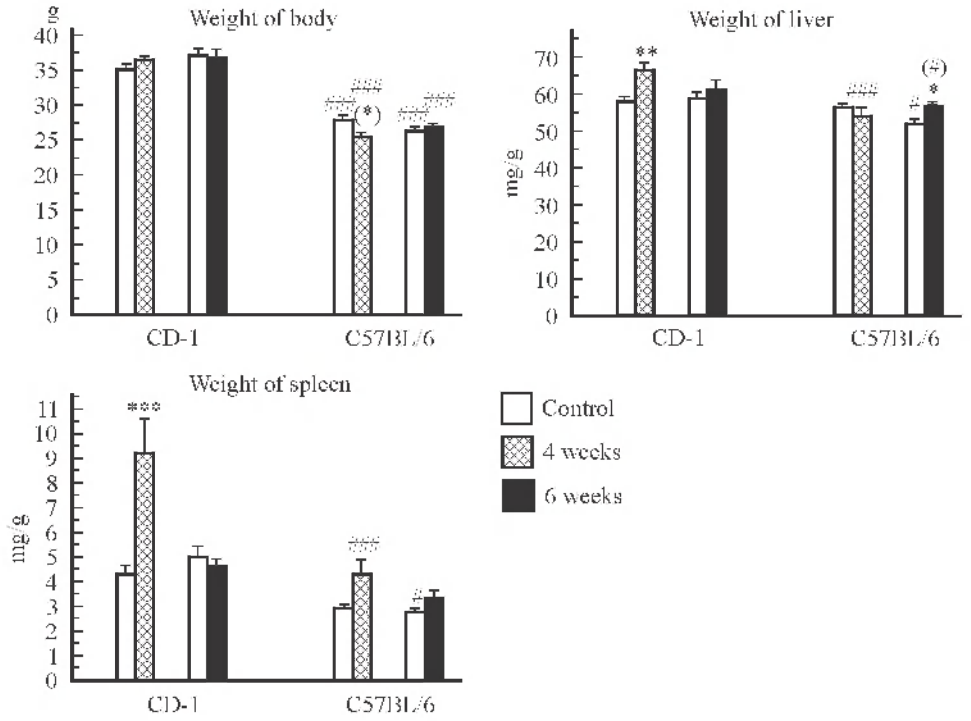

Fig. 2. Changes in body weight and in relative weights of the liver and spleen in mice of the two strains 4 and 6 weeks postinfection with *O. felineus*.

* — $p < 0.05$, *** — $p < 0.001$, $^{(*)}$ — $0.05 < p < 0.1$ (an insignificant trend) — compared to the control (for each group); $^{\#}$ — $p < 0.05$, $^{\#\#\#}$ — $p < 0.001$, $^{(\#)}$ — $0.05 < p < 0.1$ — compared to the same parameter in C57BL/6 mice.

was 2.5-fold higher than that in the control. In CD-1 mice, this parameter was noticeably but insignificantly increased at both time points: 1.7-fold after 4 weeks and 1.6-fold after 6 weeks. As shown in table 3, there was a substantial influence of factor «Group» on this parameter.

The effect of *O. felineus* infection on AST was observed in CD-1 mice after 4 weeks (fig. 3; table 3). This parameter normalized at 6 weeks in these mice. In C57BL/6 mice, there was no influence of *O. felineus* infection on AST activity

Table 2

Results of the three-way ANOVA applied to data on relative weights of the liver and spleen in mice of the two strains

Parameters	Factors			Factors' interaction
	Strain	Duration	Group	Strain*Duration*Group
Weight of body	$F_{(1.80)} = 248.80$ $p < 0.001$	$F_{(1.80)} = 0.70$ $p > 0.05$	$F_{(1.80)} = 0.17$ $p > 0.05$	$F_{(1.80)} = 3.43$ $p > 0.05$
Relative weight of liver	$F_{(1.80)} = 23.52$ $p < 0.001$	$F_{(1.80)} = 1.76$ $p > 0.05$	$F_{(1.80)} = 0.88$ $p < 0.010$	$F_{(1.80)} = 7.39$ $p < 0.01$
Relative weight of spleen	$F_{(1.80)} = 31.77$ $p < 0.001$	$F_{(1.80)} = 8.77$ $p < 0.01$	$F_{(1.80)} = 14.53$ $p < 0.001$	$F_{(1.80)} = 7.23$ $p < 0.01$

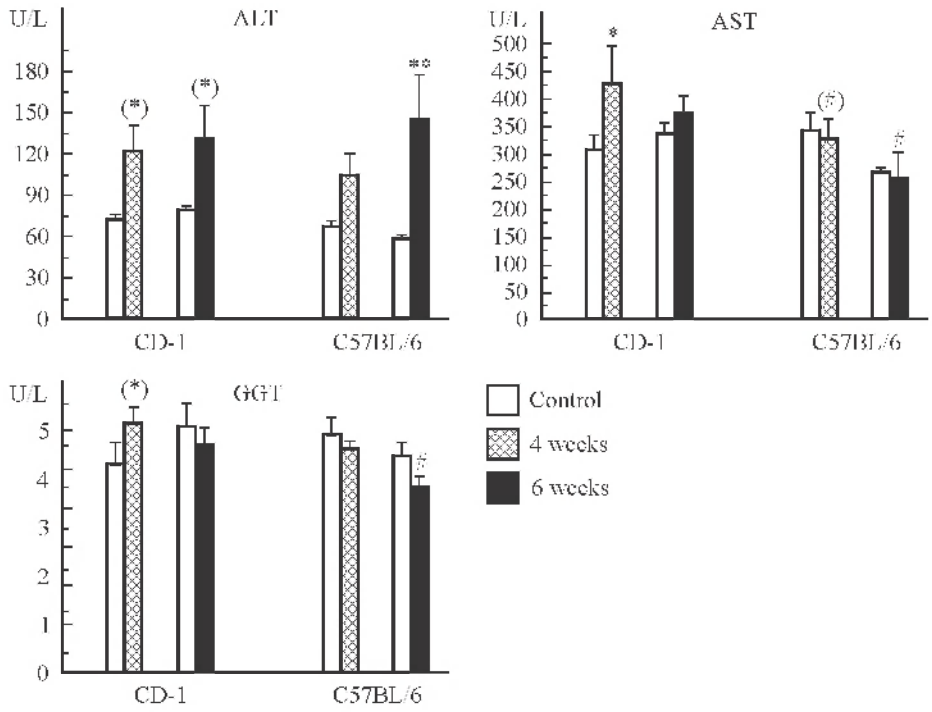

Fig. 3. Serum ALT, AST, and GGT activities 4 and 6 weeks postinfection with *O. felineus*.

* — $p < 0.05$, ** — $p < 0.01$, (*) — $0.05 < p < 0.1$ (an insignificant trend) — compared to the control (for each group); # — $p < 0.05$, (#) — $0.05 < p < 0.1$ (an insignificant trend) — compared to the same parameter in C57BL/6 mice.

at either time point. Interstrain differences in this parameter were observed during the infection: an insignificant trend after 4 weeks and statistically significant differences after 6 weeks (fig. 3; table 3).

Blood activity of GGT was somewhat increased in CD-1 mice 4 weeks after injection of *O. felineus* larvae and did not differ from the control level at 6 weeks postinfection. The infection did not affect this parameter in C57BL/6 mice during the study period. Six weeks postinfection in C57BL/6 mice, this parameter was significantly lower than that in CD-1 mice.

Table 3

The results of three-way ANOVA applied to data of the assays of biochemical parameters in mice of the two strains

Parameters	Factors			Factors' interaction
	Strain	Duration	Group	Strain*Duration*Group
ALT	$F_{(1.80)} = 0.30$ $p > 0.05$	$F_{(1.80)} = 0.69$ $p > 0.05$	$F_{(1.80)} = 16.74$ $p < 0.001$	$F_{(1.80)} = 0.80$ $p > 0.05$
AST	$F_{(1.80)} = 5.06$ $p < 0.05$	$F_{(1.80)} = 2.31$ $p > 0.05$	$F_{(1.80)} = 1.14$ $p > 0.05$	$F_{(1.80)} = 0.59$ $p > 0.05$
GGT	$F_{(1.80)} = 2.10$ $p > 0.05$	$F_{(1.80)} = 0.91$ $p > 0.05$	$F_{(1.80)} = 0.20$ $p > 0.05$	$F_{(1.80)} = 0.73$ $p > 0.05$

DISCUSSION

According to results, mice are susceptible to *O. felineus* infection, as other mammals. Their susceptibility mostly depends on the genotype of mice. For instance, in mice of inbred strain C57BL/6, the number of maritae in hepatic bile ducts was substantially higher than in outbred CD-1 mice both 4 and 6 weeks postinfection. Moreover, as we showed previously (Avgustinovich et al., 2017), the number of maritae at an even earlier time point, 2 weeks postinfection, in hepatic bile ducts of C57BL/6 mice was greater (29.69 ± 2.92) than after 4 and 6 weeks (in the present study). In mice of both strains, the maritae were found to be immature at all the analyzed time points. Other investigators also reported that in C57BL/6 mice, there are no mature maritae with well-developed reproductive organs even at 2 months postinjection with *O. felineus* larvae, although in mice of other inbred strains (CBA/Lac, A/He, and DBA/2J) mature maritae are present under these settings (Zelentsov, 1974). Our results are suggestive of varied susceptibility of the mammalian body to infection with *O. felineus*, depending on genetic factors, and apparently, outbred CD-1 mice are noticeably more resistant to this infection. This conclusion is in good agreement with the notion that inbreeding can weaken resistance to pathogens and parasites (Ilmonen et al., 2008; Hofer et al., 2010). In this regard, the resistance of outbred CD-1 mice to infection with *O. felineus* manifests itself mostly as well-pronounced elimination of maritae from hepatic bile ducts.

Besides, in outbred CD-1 mice, there was an increase in relative weight of the liver and especially of the spleen, 4 weeks postinfection. These parameters returned to normal values after 6 weeks postinfection. In inbred C57BL/6 mice, we observed an increase in liver weight 6 weeks postinfection. The relative spleen weight in C57BL/6 mice did not change during the study period although, as we showed previously (Avgustinovich et al., 2017), it is substantially enlarged 2 weeks postinfection. The spleen is considered the biggest organ of the peripheral immune system. As pointed out by some researchers, acute opisthorchiasis caused by *O. felineus* is characterized by hepatosplenomegaly (Furst et al., 2012). Similarly, our results indicate splenomegaly in mice of the two strains, but in outbred CD-1 mice, this condition persists longer (week 4) than in C57BL/6 mice (week 2). These data imply longer persistence of the elevated activity of the immune system in outbred mice. It was demonstrated recently that outbred CD-1 mice display a more pronounced proinflammatory response (than inbred C57BL/6 mice do) to centrally administered lipopolysaccharide (LPS) (Nikodemova, Watters, 2011). Apparently, invasion of bile ducts by *O. felineus* larvae in CD-1 mice also causes a stronger inflammatory reaction in comparison with C57BL/6 mice. It is possible that the leading role in these processes is played by macrophages, which derive from maturing monocytes in the spleen, whose relative weight in outbred CD-1 mice stays elevated until 4 weeks postinfection. This hypothesis is supported by the data from outbred ICR mice (compared to inbred CBA mice), showing more pronounced activation of granulopoiesis of the myeloid lineage in response to specific and nonspecific stimuli (Hofer et al., 2010). Those authors concluded that inbreeding inhibits the granulopoietic system in mice.

Nonetheless, another explanation cannot be ruled out either, namely, that the dose of *O. felineus* metacercariae used in the present study (100 larvae per mou-

se) is not sufficient to induce the development of opisthorchiasis in outbred CD-1 mice. It is known, for example, that 3 days after intravenous inoculation of the malarial parasite *P. berghei* at the dose of 50 sporozoites per animal, these parasites are detected in all the injected mice of inbred strain C57BL/6, whereas in outbred CD-1 mice, the parasites are undetectable (Scheller et al., 1994). In contrast, at the dose of 20.000 sporozoites, the malarial parasites are detected in 100 % of cases in both strains of mice. Apparently, the functional abilities of the immune system in outbred CD-1 mice substantially depend on the strength of the infectious agent, which is determined by recurrence of infection or by the dose of the invading metacercariae. It is possible that at higher doses of *O. felineus* larvae, the modeling of opisthorchiasis in this strain of mice will be more successful. It may happen that creation of an opisthorchiasis model by means of CD-1 mice will require additional (two- to three-time) infection with *O. felineus* larvae. In any case, in golden hamsters (which are most frequently used as model animals in experiments with *O. felineus*-induced opisthorchiasis), superinvasions of the parasite, especially at the stage of chronic opisthorchiasis (when exhaustion of defense reserves of the host organism is observed) cause a threefold increase in the number of helminthes in the hepatobiliary system (Stepanova, Podkletnova, 2002).

Despite the insignificant number of *O. felineus* maritae in hepatic bile ducts of CD-1 mice ($\leq$ 4% of the injected metacercariae) and the prepatent period of their development (in terms of their maturity), these maritae have an appreciable negative effect, judging by the biochemical parameters in blood. There was a significant increase in AST and GGT activities (4 weeks postinfection) and somewhat increased activity of ALT (at both time points under study) in blood serum. It is known that ALT is located in the cytoplasm of hepatocytes, AST serves as a mitochondrial-cytoplasmic enzyme, whereas GGT is a membrane-bound enzyme (Kishkun, 2013). Consequently, the increase in activity of these enzymes reflects processes of hepatic cytolysis, which can take place in response to various stressors (for the body as a whole), including infectious agents. A challenge with LPS causes a significant increase in the activities of serum ALT and AST in rats (Bharrhan et al., 2010). Infection with *Schistosoma mansoni* Sambon, 1907 induces significant elevation of serum levels of GGT (+343.44 %), AST (+209.19 %), ALT (+157.10 %), and alkaline phosphatase (ALP) (+478.94 %) as compared to healthy control mice (Aly, Mantawy, 2013). Upregulation of ALT and/or AST as well as GGT was demonstrated during acute opisthorchiasis in silver foxes (Schuster et al., 2003) and in humans (Bakshtanovskaia et al., 1996; Bronstein, Luchshev, 1998; Bakshtanovskaia et al., 2003).

In clinical practice (in humans), there are these notions of intrahepatic and extrahepatic cholestasis, associated with disturbances of bile outflow from small and large bile ducts, respectively, in the liver (Kishkun, 2013). Furthermore, an increase in GGT activity is the most reliable marker of intrahepatic cholestasis. We can hypothesize that in outbred CD-1 mice, *O. felineus* maritae cause the development of liver pathology under the scenario of intrahepatic cholestasis. Nonetheless, by the time point 6 weeks, strong regenerative abilities of the liver return these parameters to normal levels. Even though the enzymatic activity of AST increased statistically significantly, this increase was less than severalfold. Besides, this parameter also normalized after 6 weeks.

In contrast to outbred CD-1 mice, in inbred C57BL/6 mice, activity of ALT turned out to be strongly elevated 6 weeks postinfection. Probably, in mice of this strain, continuation of opisthorchiasis development (chronic opisthorchiasis) is a more expected phenomenon than in outbred CD-1 mice. Testing of this theory, however, would require further comparative studies on the consequences of infection in inbred C57BL/6 and outbred CD-1 mice at more distant time points postinfection with *O. felineus*.

In conclusion, it should be mentioned that after a one-time injection of *O. felineus* metacercariae in the amount of 100 larvae per mouse, we found inbred C57BL/6 mice to be more susceptible to active infection than CD-1 mice were. Greater presence of juvenile worms was detected in the hepatobiliary system of the inbred mice 4 and 6 weeks postinfection, as was increased weight of the liver and enhanced ALT activity in blood after 6 weeks. These and previously reported data (Avgustinovich et al., 2016; Avgustinovich et al., 2017), support the advantages of inbred C57BL/6 mice as an experimental model for further research into processes of the development of experimental opisthorchiasis. It should be emphasized, however, that the resistance of outbred CD-1 mice to *O. felineus* infection is a major independent finding of the present study because it should help to uncover the mechanisms underlying the confrontation between the mammalian body and a parasitic infection.

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