

The duration of rest and feeding greatly affects the re-breeding of ectoparasites: *Hirudo verbana*, *Hirudo medicinalis* and *Hirudo orientalis*

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ABSTRACT. Ectoparasitic leeches have many biologically active substances in their body, which are able to show various therapeutic effects, which makes them very relevant in the study. Among them, the most common are medicinal leeches: *Hirudo verbana*, *Hirudo medicinalis* and *Hirudo orientalis*. They are listed in the Red Book as a vulnerable species, so their population is mostly supported in biolabs. Therefore, the search for different methods of their preservation is relevant. The aim of the work was to test the effect of the duration of rest and feeding of animals for re-reproduction, which will increase their population. For the study, three experimental animal groups were formed: 1 control – the animals were fed a week after the first reproduction, re-reproduction occurred not earlier than 2 months; 2 experimental – selected animals that remained clitellum after the first reproduction and again sent to the peat-soil environment for reproduction without rest and feeding; 3 experimental – selected animals that remained clitellum after the first reproduction, fed after 1 week, after another 1–2 weeks again sent to the peat-soil medium for reproduction. As a result of the study, the restoration of the physiological state after the first dilution in the first and third groups was registered. In the second group, the animals are depleted as a result of defective offspring, mortality of them and their offspring.

Keywords: leeches, parasite, ectoparasitism, *Hirudo*, reproduction

Introduction

Ectoparasitic medicinal leeches have long been used in veterinary and medicine because they have healing properties for various ailments. The first information about the use of leeches for medical purposes dates back to Ancient Egypt. Fragments of true paintings depicting the jubilation of leeches were found in the tombs of the pharaohs of the 18th dynasty. According to known facts, they parasitise on the surface or in the cavities near to the surface of the body. But there are leeches that have in their body many biologically active substances that can exhibit various therapeutic effects [1–15]. Among the most common and studied medicinal leeches are *Hirudo verbana* Carena, 1820; *Hirudo medicinalis* Linnaeus, 1758 and *Hirudo orientalis* Utevsky and Trontelj, 2005. These three species are used for the prevention and treatment of many diseases, are also effectively studied in oncology, tuberculosis,

recovery after Covid-19 and others [2,3,5,6,8–12]. But in nature, these animals are almost extinct, and they are listed in the Red Book as a vulnerable species, so their population is mostly supported in biolaboratories. Scientists are looking for different methods to maintain them and increase their population. For example, we and other scientists have proved that the temperature in the range of +22–25°C, is the most optimal for reproduction and deposition of cocoons, and the pH environment solution is in the range of 5.0–6.5 for normal life [16–21]. The normal weight of adult animals is 5.94±1.06 g, and the best for breeding humidity of peat is about 70–75% [22,23]. The efficiency of normalization of life and increased productivity of animals during feeding of various domestic animals is proved [24]. The optimal age of animals for reproduction and obtaining a large number of offspring is shown [25]. According to scientific data of other scientists, it is not shown after what time

Table 1. Mortality of adult animals and their offspring, cocoon defect

Indicators	The first group			The second group			The third group		
	<i>H. verbana</i>	<i>H. medicinalis</i>	<i>H. orientalis</i>	<i>H. verbana</i>	<i>H. medicinalis</i>	<i>H. orientalis</i>	<i>H. verbana</i>	<i>H. medicinalis</i>	<i>H. orientalis</i>
Mortality of adult animals,%	1±0.3	1.6±0.3	1.4±0.2	4±0.2*	6±0.5*	5.5±0.4*	1.6±0.3	2.3±0.4	1.9±0.3
The average number of cocoons per animal, pieces	9.5±1.5	5.1±0.9	7.2±0.8	5±0.7*	3.5±0.5*	4±0.3*	8.5±0.6	4.2±0.4	6.5±0.4
Not fertile cocoons, pieces	1.9±0.3	2.1±0.2	2.1±0.1	3±0.2*	4±0.3*	3.6±0.2*	2.2±0.1	2.5±0.2	2.4±0.2
Defective cocoons, pieces	1.5±0.1	2.1±0.1	1.9±0.1	2.5±0.1*	2.9±0.1*2	.9±0.1*	1.7±0.2	2.2±0.1	2.0±0.1
Defective young animals,%	0.9±0.2	1.8±0.3	1.2±0.2	10±0.9*	15±1.3*	12.6±1.4*	1±0.3	2.3±0.3	1.7±0.3
Dead young animals,%	0.8±0.2	1.2±0.3	1.1±0.2	2.3±0.2*	3.6±0.4*	3.5±0.3*	1.2±0.3	1.4±0.3	1.2±0.2

I* – $P < 0.05$ in comparison with the control group

leeches are able to recover in artificial conditions and are able to lay cocoons again. What will make it possible to increase the population without harming

them. Therefore, the aim of the work was to test the effect of the duration of rest and feeding of animals for re-reproduction.

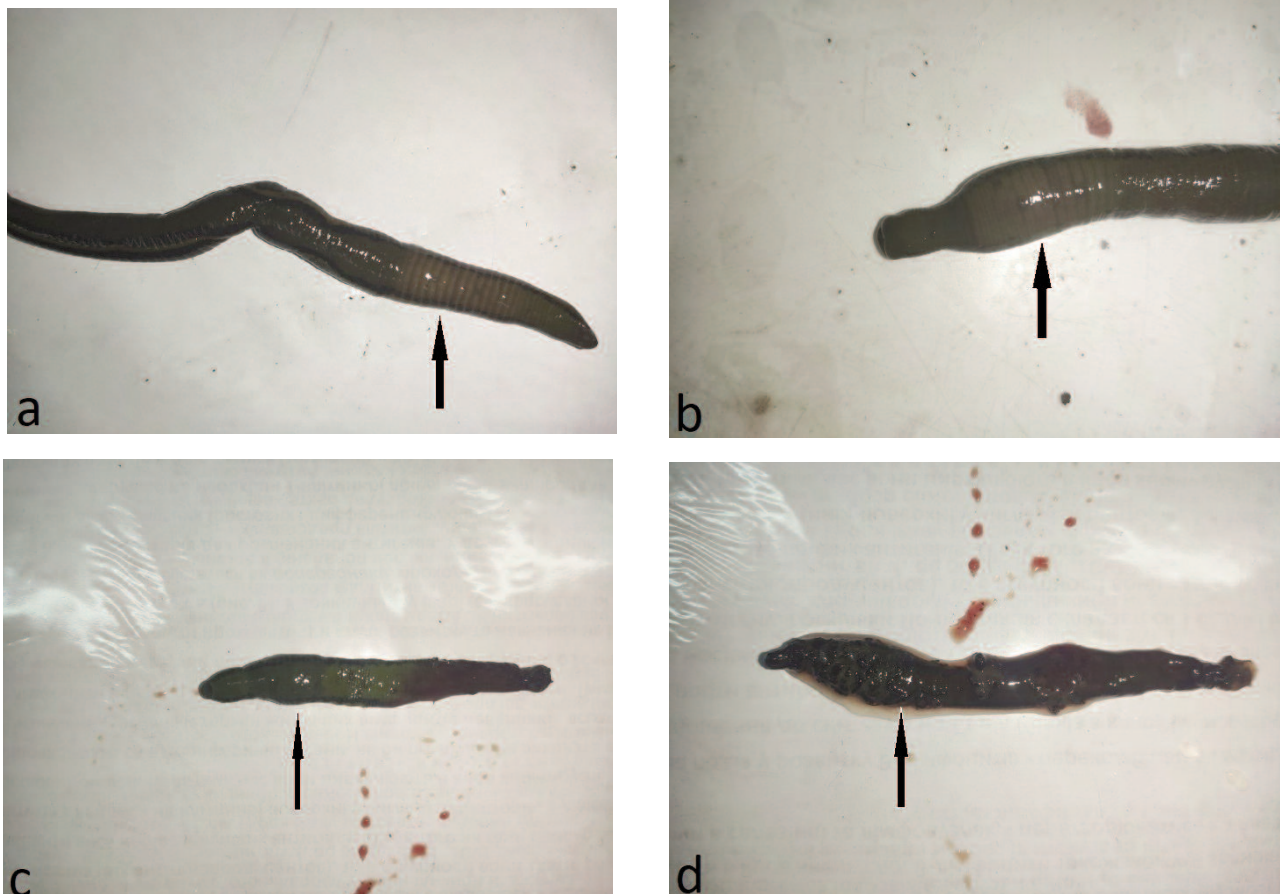


Figure 1. Dead medical leeches *Hirudo verbana* Carena, 1820, with clitellum (indicated with an arrow); a and b. without visual damage to the body; c and d. visually damaged body

Materials and Methods

Group of animals

The study used 90 animals of medicinal leech ectoparasites of each species *Hirudo verbana* Carena, 1820; *Hirudo medicinalis* Linnaeus, 1758 and *Hirudo orientalis* Utevsky and Trontelj, 2005. For the study, three experimental animal groups were formed: 1 control – animals after the first reproduction, a week later fed on the blood of domestic animals, re-reproduction occurred not earlier than 2 months. The process of feeding medicinal leeches with blood: the small intestine is filled with the blood of a domestic animal. Blood and intestines were from animals genus *Sus*. The feeding process of medicinal leeches was similar to natural conditions (blood temperature (+37–39°C) and environment (+20°C) where it was during feeding, small intestine instead of skin); 2 experimental – selected animals that remained clitellum after the first reproduction and again sent to the peat-soil environment for reproduction without rest and feeding; 3 experimental – selected animals that remained clitellum after the first reproduction, fed after 1 week, after another 1–2 weeks again sent to the peat-soil medium for reproduction. The control group of animals was re-tested in reproduction after two months, statistically it did not differ from the first reproduction.

Experiment scheme

Animals with clitellum after copulation (6–8 pieces) in the aquatic environment, two weeks after feeding on the blood of a domestic animal were sent to the peat-soil environment for reproduction. All conditions are made and tested according to the experiments of other scientists and our experiments [16–25]. Up to 1 months, they were in this environment. At a room temperature of +25–26°C, diffused daylight, severe silence and frequent air ventilation. After the appearance and final formation of cocoons, adult animals of all groups were removed. Further studies were conducted according to the distribution of groups. The mortality of adult animals, the average number of cocoons per animal, the defect of cocoons and young animals were studied.

Bioethics

Animal manipulation was carried out in accordance with the rules and regulations for the treatment of laboratory animals: principles of bioethics, legislation and requirements in

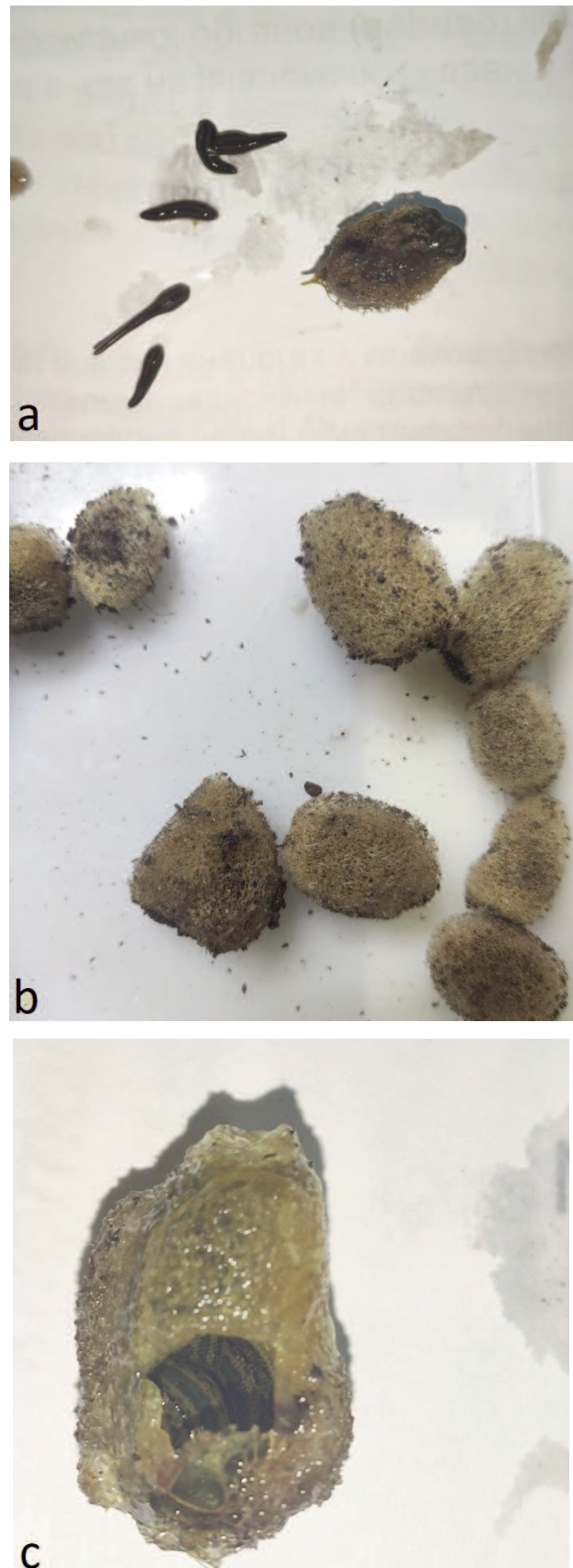


Figure 2. Physiologically normal cocoons (after 1 month): a. *Hirudo orientalis* Utevsky and Trontelj, 2005; b. *Hirudo medicinalis* Linnaeus, 1758; c. *Hirudo verbana* Carena, 1820

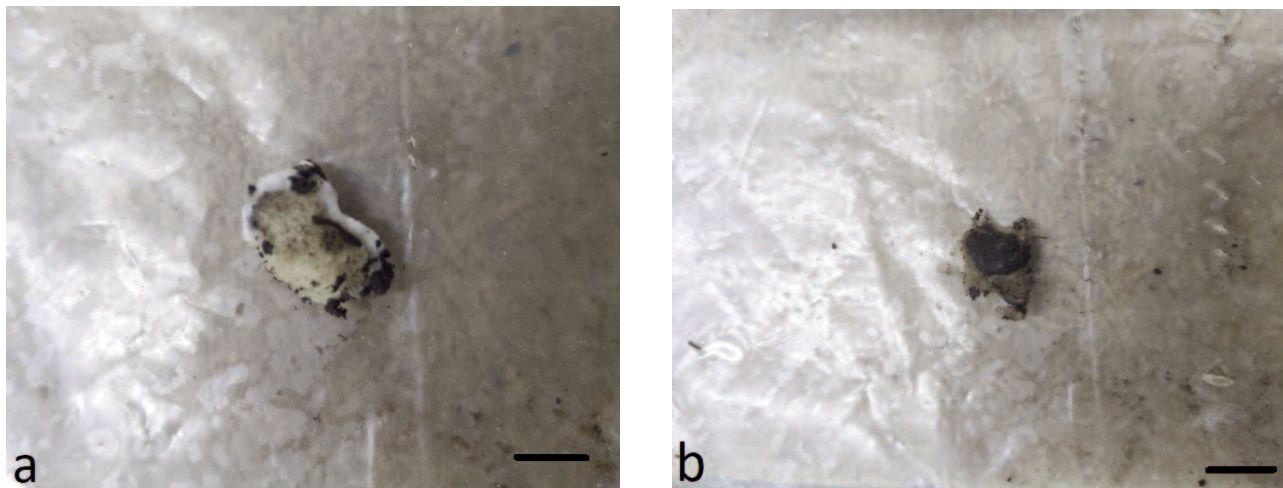


Figure 3. Defective cocoons (after 1 month) *Hirudo verbana* Carena, 1820; a and b. Scale bars 0.5 cm

accordance with the provisions of the „European Convention for the Protection of Vertebrate Animals Used for Research and Scientific Purposes”, the Law of Ukraine „On the Protection of Animals from Animals handling”. Such a responsible commission received from the bioethicists of Zaporizhzhya National University protocol №1 from March 28, 2018.

Statistical analysis

Statistical data processing was performed using the computer program SPSS v.23,0. (IBM SPSS Statistics., USA). The selected parameters indicated in the table below have the following notation: X – the average value of the sample, SE – standard error of the average value of the sample. The significance of differences between the mean values was evaluated by the Student’s criterion after checking the normal distribution. Differences were considered significant at $P < 0.05$.

Results

When analyzing the mortality rate of adult animals, we found that the third group of all three species *Hirudo verbana* ($1.6 \pm 0.3\%$), *Hirudo medicinalis* ($2.3 \pm 0.4\%$) and *Hirudo orientalis* ($1.9 \pm 0.3\%$) did not differ statistically from the first group ($1 \pm 0.3\%$, $1.6 \pm 0.3\%$, $1.4 \pm 0.2\%$, respectively) (Tab. 1). In the analysis of the second group in comparison with the first and third, we observed increased mortality ($4 \pm 0.2\%$, $6 \pm 0.5\%$, $5.5 \pm 0.4\%$, respectively). Animals were dying, and fertility belts were visible on their bodies (Fig. 1). The average number of cocoons obtained per animal did not differ statistically in the first group compared

with the third (Tab. 1). Another picture we observed in the second group when compared to others there was a significant decrease in the number of cocoons. It should be noted that the cocoons of all groups did not differ (Fig. 2). But in the second group there were more non-fertile and defective cocoons compared to other groups of (Tab. 1, Fig. 3). In the analysis of young animals, we also see a significant percentage of defective immature leeches or dead before and after the first feeding in comparison with other groups of (Tab. 1, Fig. 4).

Discussion

As a result of the study, we see that the duration of rest and feeding of animals for re-reproduction of animals plays a significant role in their lives and reproduction. When analyzing the mortality of adult animals, we can see that the animals of the second group are the most favorable compared to the first and third groups where the animals had time to rest and feed on the blood of a domestic animal. This is indicated by our previous studies and studies of other scientists, who indicate that the disturbed physiological state of animals has a very negative impact on their lives [19–25]. But according to the results, we observe not only a violation of their physiological state, but also their reproductive system, which is reflected in their offspring: a decrease in the average number of cocoons, their defects and defects of the offspring. In the first and third groups, we did not observe significant deviations, which indicates the normalization of the physiological state of the animal, due to rest and feeding of the animal. Using the method of breeding animals, as in the first and third groups, we can

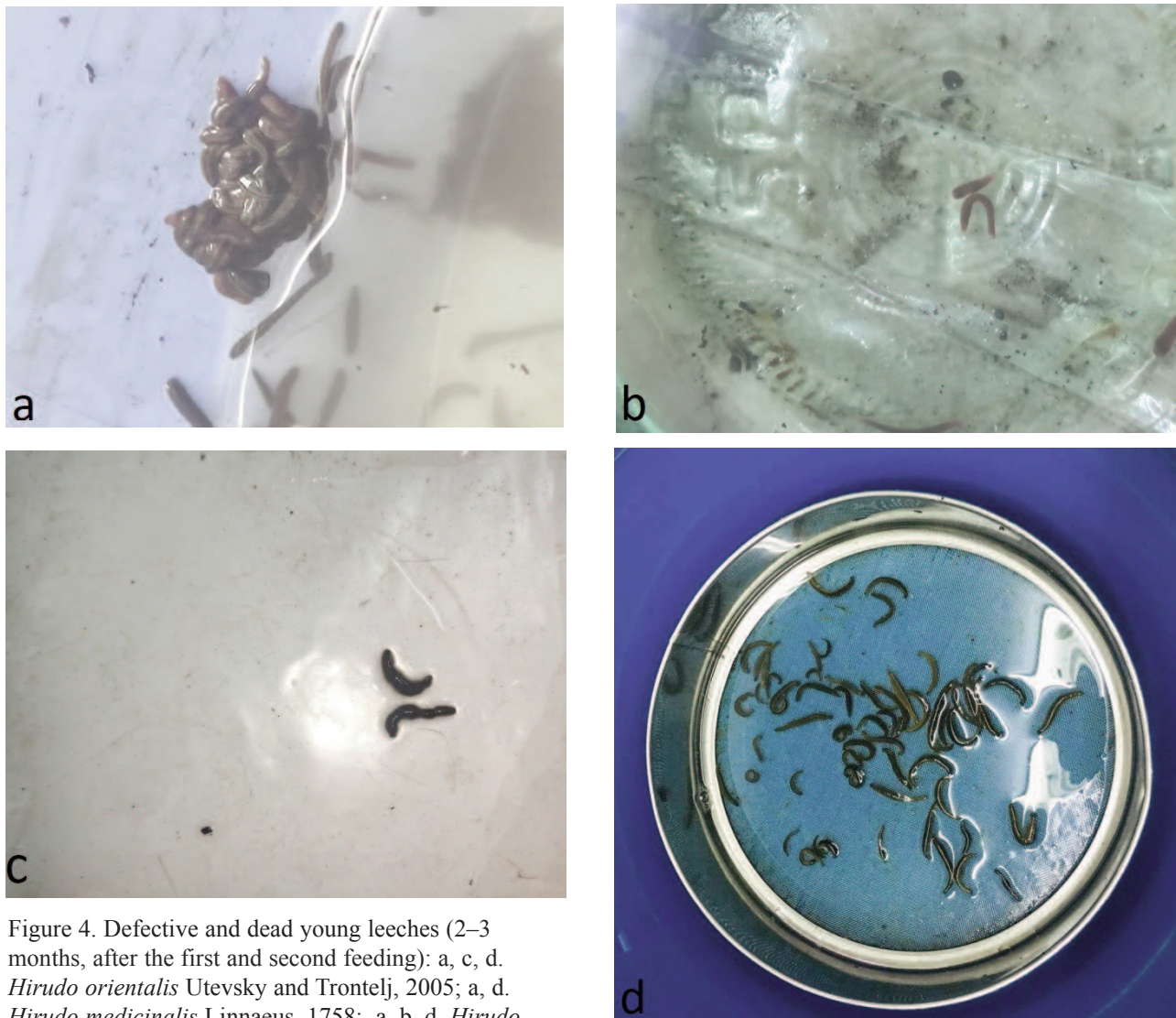


Figure 4. Defective and dead young leeches (2–3 months, after the first and second feeding): a, c, d. *Hirudo orientalis* Utevsky and Trontelj, 2005; a, d. *Hirudo medicinalis* Linnaeus, 1758; a, b, d. *Hirudo verbana* Carena, 1820

increase the population of their species. The third method can be used if you want to quickly increase the number of animals, and time is short. In general, the analysis of the second group indicates the deterioration of the general condition of the animal as a result of depletion of the animal after the first reproduction. It is not recommended to re-send animals for breeding without rest and feeding. Because it can cause the death of them and their offspring. The findings will be useful for all scientists studying ectoparasitic medical leeches.

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