Annals of Parasitology 2025, 71, 79–83
doi: 10.17420/ap71.549

Original paper

Optimal artificial conditions for keeping and breeding medicinal leeches *Hirudo verbana* and *Hirudo orientalis*

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ABSTRACT. The aim of the work is to obtain optimal artificial conditions for the maintenance and breeding of medicinal leeches of the species *Hirudo verbana* and *Hirudo orientalis*. 180 medicinal leeches were used in the study: 100 leeches of the *H. verbana* species and 80 *H. orientalis* leeches. Four groups of animals were formed: two control and two experimental. Control groups of medicinal leeches were kept using the standard jar method. Experiments according to the developed optimal scheme. As a result of the study, it was found that there were more juveniles obtained from the cocoon per 1 parent leech in the experimental group of medicinal leeches of the *H. verbana* and *H. orientalis* species, compared to the control group; appearance of defective cocoons. Mortality of parent leeches during breeding, mortality of juveniles before the first, after the first and sixth feeding decreases on average by more than 50% ($P < 0.05$) in the experimental group.

Keywords: medicinal leeches, optimal environment, breeding, keeping.

Introduction

Medicinal leeches are ectoparasitic animals that can show a significant amount of therapeutic effects through the presence of biologically active substances in their body [1–21]. There are scientific references that medicinal leeches can serve as bioindicators of the aquatic environment. Because they respond to minor changes in environmental fluctuations. As a result, many types of leeches disappear or disappear in natural conditions. For example, scientists discovered the mortality of leeches in reservoirs that were contaminated with heavy metals Zn and Cu [22]. Various concentrations of zinc and copper with long-term use can cause death and uncontrolled defecation in animals, intense secretion of mucus, uncontrolled swimming, reduced grip strength, twisting, vomiting of eaten blood, body deformation, penile prolapse, hemorrhages and abrasion of the body wall in *H. verbana* Carena, 1820 [22]. Water quality parameters such as water temperature, dissolved oxygen, pH, some cationic elements and anionic elements can significantly affect the viability of leeches [22–24].

In the works of some scientists, the optimal temperature conditions for mating and laying cocoons are established: +22–25°C and +24°C, respectively. At a temperature below +15–16°C, copulation does not occur. It has been established that a weakly acidic (pH 5.0–6.5) environment is optimal for the normal life activity of medicinal leeches. The weight and blood of medicinal leeches can significantly affect the quality of offspring [25–34]. Air ventilation inside the cocoons is more efficient when incubated in a vertical position, compared to cocoons that are incubated in a horizontal position. Therefore, to obtain a larger number of offspring, it is necessary to incubate the cocoons at an angle close to the vertical, more than

60°C [35]. When feeding mature leeches of the species *H. orientalis* with different types of frog and camel blood, it was found that the number of fertile full-fledged cocoons and offspring is significantly higher when fed with camel blood, compared to when fed with frog blood. Mortality of leeches is most observed when fed with frog blood, compared to when fed with camel blood [34]. According to the conducted studies, there are many different methods of keeping medicinal leeches by changing the environmental conditions, but the majority use exactly three well-known methods of keeping leeches [36]:

1. Classical method is to add stones and dirt, aquatic plants such as Java moss and Microsorumpteropu to clay vessels.

2. Modern jar method is the use of plastic or glass jars with holes in the lids for ventilation.

3. Aquarium method – using aquariums with water, dry branches and aquatic plants.

However, these methods of artificial maintenance are not fully effective and not standardized. They require improvements and clarifications.

Therefore, the aim of the work was to obtain optimal artificial conditions for the maintenance and breeding of medicinal leeches of *H. verbena* and *H. orientalis* species.

Materials and Methods

Group of animals

180 medicinal leeches were used in the study: 100 leeches of the *H. verbana* species and 80 *H. orientalis* leeches. Four groups of animals were formed: two control and two experimental. The control groups of animals were medicinal leeches of both species, which were kept using the modern jar method. Grown on the basis of the educational-scientific-research laboratory of cellular and organismal biotechnology of Zaporizhzhya National University (TU U 05.0-02125243-002:2009 "Medicinal leech", sanitary-epidemiological opinion of the Ministry of Health of Ukraine No. 05.03.02-06/49982, dated 12.08 .2009). Conditions of experiment are described in next chapter.

Experiment scheme

1. First, a peat-soil weakly acidic environment (pH 6–6.5) was prepared: the prepared natural soil underwent visual detailed selection (for pests and other animal organisms) and disinfection (using high temperatures), then it was moistened with distilled water (70–85%), mixed thoroughly, then diluted with neutral peat in a ratio of 1:3, mixed and grinded. In the future, it was covered with an airtight tablecloth for at least 5 days.

2. Experimental medicinal leeches (6 animals per container) were placed in sterile 3-liter glass containers with tap settled water with a room temperature of + 25–27°C and diffused natural and artificial lighting. Leeches were fed 3 times with the blood of cattle filled into the healthy intestines of these cattle, heated to a temperature of + 37–39°C. During the first 48 hours, the water environment of their keeping was changed twice a day. Further, water was changed every three days. As fertilization belts formed in leeches, they were transplanted (4 leeches per container) into 3-liter sterile containers with a moist peat-soil medium. Holes were artificially made in a vessel with a peat-soil environment, a leeches were placed in them and they were watered with settled water. After that, the containers were covered with a fabric capable of fully permeable oxygen, and the containers with the animals were placed in a dark room with a temperature of + 25–26°C and diffused artificial lighting. Strict control over the humidity of the environment, room temperature, constant ventilation and oxygen supply was maintained. After a month, parent leeches and soft cocoons were collected from which juveniles were selected and placed in sterile 3-liter containers with settled tap water, in a room with room temperature and diffused lighting. Solid cocoons in the same peat-soil medium were left to incubate for a month in a horizontal position, with detailed inspection once a week. Since scientists have proven that the horizontal position of the cocoons makes it possible to reduce the formation of suppuration in them and increase the percentage of obtaining viable offspring.

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 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".

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 and Discussion

According to the indicators from Table 1, in the experimental group of *H. verbana*, more juveniles are obtained from a cocoon per 1 leech compared to the control group by an average of 19.0% ($P < 0.05$), the appearance of defective cocoons decreases by 60.8%, mortality of female leeches during breeding decreases by 20.8%, mortality of juveniles before first feeding decreases by 58.7% ($P < 0.05$), mortality of juveniles after first feeding decreases by 65.4% ($P < 0.05$), mortality of juveniles after the sixth feeding decreases by 33.4% ($P < 0.05$).

Similar results are observed when analyzing medicinal leeches of the species *H. orientalis* (Table 1). More juveniles are obtained from a cocoon per 1 parent leech compared to the control group by an average of 29.6% ($P < 0.05$), the appearance of defective cocoons decreases by 65.4%, the mortality of parent leeches decreases by an average of 60% ($P < 0.05$). It should be noted that the mortality of juveniles significantly decreases in all groups after repeated feeding. Perhaps the animals adapt to the consumed blood, which is also confirmed by scientists showing the effectiveness of the blood of cattle when feeding medicinal leeches [23,25,27,29,30,32–34]. In our previous studies, we showed that the blood type, as well as arious endogenous and exogenous factors in the maintenance and breeding of medicinal leeches can negatively affect adult leeches and their offspring [29,37,38]. Therefore, in this study, medicinal leeches from possible exclusion of these negative factors.

Summarizing the obtained results regarding the indicators of the control group in omparison with the experimental group, indicate that the optimal conditions for keeping and breeding medicinal leeches were selected in the experimental group.

Table 1. Indicators of the experimental group in comparison with the experimental group (X ± SE; n=180)

Group of animals	Average number of juveniles obtained from a cocoon per 1 parent leech	Mortality of parent leeches during breeding, %	Defective and non-fertile cocoons, %	Mortality of juveniles, before the first feeding %	Mortality of juveniles, after the first feeding %	Mortality of juveniles, after the sixth feeding %
Control group (modern banking method) H. verbana (n=50)	8.8±0.4	2.3±0.1	5.3±0.3	4.5±0.2	2.5±0.2	1.4±0.1
Experimental group H. verbana (n=50)	10.5±0.3*	1.5±0.2*	2.1±0.3*	1.8±0.2*	0.8±0.1*	1.1±0.2*
Control group (modern banking method) H. orientalis (n=40)	5.7±0.1	2.6±0.2	5.7±0.2	5.1±0.2	3.0±0.3	1.8±0.2
Experimental group H. orientalis (n=40)	8.1±0.2*	1.7±0.2*	3.3±0.3*	2.1±0.2*	1.1±0.1*	1.2±0.1*

Explanation: * – P < 0.05 compared to the control group

Also, the selected ratio of peat and soil is optimal for the environment.

Acknowledgements

This work was supported by the Laboratory of Cell and Organism Biotechnology of Zaporizhzhya National University.

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Received 25 February 2024
Accepted 19 July 2025