

Original paper

The effect of blood human, domestic and small laboratory animals on the viability and behavior of the medicinal leech

Ruslan AMINOV

Cellular and Organism Biotechnology Laboratory, Faculty of Biology, Zaporizhzhia National University, Zhukovsky Street 66, 69600 Ukraine

E-mail: 91_amin_91@ukr.net

ABSTRACT. Medicinal leeches are used for therapeutic purposes in the prevention and treatment of many diseases. Because they have a large amount of biologically active substances in their body. Each of these substances has many therapeutic effects. In natural conditions, they are mostly fed of blood by wild animals. In laboratory conditions, the blood of domestic animals is mostly used. Currently, medicinal leeches are mostly bred in laboratory conditions. Because there are very few of them in nature. They are listed in the Red Book. Scientists of various specialties are looking for optimal conditions for their life and breeding. That became our research goal. To identify the influence of blood human, domestic animals (pigs and chickens) and small laboratory animals (rats) on the viability and behavior of medicinal leeches *Hirudo verbana* Carena, 1820 and *Hirudo orientalis* Utevsky & Trontelj, 2005. According to this, 8 groups of sexually mature animals were formed: 1 and 2 – human blood; 3, 4 – blood of a domestic pig; 5, 6 – blood of domestic chickens; 7, 8 – blood of a non-linear laboratory rat. As a result of the study, it was found that the blood of pigs and chickens is the most suitable for feeding the medical leech for normal life and behavior. Mortality of leeches was observed when feeding on rat and human blood. It should be noted that at the beginning of feeding animals with blood human. The percentage of cannibalism in animals increased.

Keywords: blood, leeches, ectoparasitism, *Hirudo*, medicinal leech

Introduction

Medicinal leeches are mostly ectoparasitic animals. Most of which are bred in biological factories and biolaboratories for medicinal use [1–10]. Since they disappear in nature, and every year their global geographical distribution decreases. They are listed in the Red Book as an endangered species. Scientists of various specialties who study them. Various methods and conditions are being sought to whiten and preserve these animals. Scientists have already proven that the optimal temperature and pH for vital activity and laying of cocoons [11–13], peat humidity [14,15]. The nutrition of sheep blood [16], the optimal age of animals for reproduction and obtaining a large number of offspring [17]. In our previous experiments, we showed that medicinal

leeches are capable of cannibalism. But the mortality of medicinal leeches remains the main problem of biolaboratories. The problem of finding optimal conditions is the main task of scientists, biofactories and biolaboratories for their preservation as a species. The blood is the main food of medicinal leeches. Which enables animals to survive in natural and laboratory conditions. But she is can be fatal for the animal. Since the adaptive properties of medicinal leeches to eaten blood are different. And in most cases, they cannot cope with the immunological factors of the eaten blood of the host. This became our goal to select more optimal blood from various vertebrates, which is mostly used in the world to feed medicinal leeches: *Hirudo verbana* Carena, 1820 and *Hirudo orientalis* Utevsky & Trontelj, 2005

Materials and Methods

Group of animals

The study used 150 animals of medicinal leech (6 months) of each species *Hirudo verbana* Carena, 1820, and *Hirudo orientalis* Utevsky & Trontelj, 2005. For the study, 8 experimental animal groups were formed: 1 (*Hirudo verbana*) and 2 (*Hirudo orientalis*) – human blood; 3 (*Hirudo verbana*), 4 (*Hirudo orientalis*) – blood of a domestic pig (*Sus domestica*); 5 (*Hirudo verbana*), 6 (*Hirudo orientalis*) – blood of domestic chickens (*Gallus gallus domesticus*); 7 (*Hirudo verbana*), 8 (*Hirudo orientalis*) – blood of a non-linear laboratory rat (*Rattus*).

Experiment scheme

Human and rat blood was obtained during suction (hirudological influence). Pig and chicken blood from the slaughterhouse. All are conditionally healthy. The feeding process of the medical leech was similar to natural conditions (blood temperature (+37) and environment (+20°C) in which it was while feeding on the blood of pigs and chickens, small intestine instead of skin). In total, the animals were fed 3 times, from the blood of 15 individuals of each species. During this time, the mortality and behavior of medical leeches of two species: *Hirudo verbana* Carena, 1820, and *Hirudo orientalis* Utevsky & Trontelj, 2005. The experiment lasted 7 months. Leeches were kept in 3-liter containers with water, 8 individuals each. Under the same lighting, temperature and humidity conditions. The control group for comparison used animals that were fed with pig's blood. Since in most biolaboratories they are fed with their blood.

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

As a result of the study, after the first feeding of 6-month-old medical leeches, it was found that *Hirudo verbana* began to die in the first and second weeks (Tab. 1): in group 1 up to 10.0±0.9%, in group 7 up to 6.1±0.6% (Tab. 1). In group 3, the mortality rate is insignificant, 0.2±0.01%, and in group 5, no mortality was detected at all (Tab. 1). It should be noted that when feeding on the blood of a rat and a human, constrictions appear on the body of leeches (in 1.1±0.07% and 4.0±0.9%, respectively) (Tab. 1). In the analysis of *Hirudo orientalis* animals, similar results were found compared to *Hirudo verbana*, but in a smaller range between the blood of a person (group 2) and rat (group 8) (6.0±0.5% and 5.2±0.7%) (Tab. 1). Constrictions on their body appeared only when fed with human blood (in 2.1±0.3%). During the feeding of leeches with pig (group 4) and chicken (group 6) blood, no

Table 1. Mortality, appearance of constrictions and manifestation of cannibalism, first feeding

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>
Mortality %	10.0±0.9*	6.0±0.5*	0.2±0.01	0	0*	0	6.1±0.6*	5.2±0.7*
Appearance of constrictions %	4.0±0.9*	2.1±0.3*	0.02±0.002	0.01±0.001	0.01±0.002	0	1.1±0.07*	0.01±0.001
Manifestations of cannibalism %	0.01±0.002*	0	0.01±0.002	0	0*	0	0.01±0.003	0

* – $P < 0.05$ in comparison with the 3 and 4 group

Table 2. Mortality, appearance of constrictions and manifestation of cannibalism, second feeding

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo . orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>
Mortality %	8.3±0.6*	0.2±0.03*	0.02±0.001	0	0.01±0.001	0	4.0±0.3*	0.2±0.01*
Appearance of constrictions %	4.0±0.5*	0.3±0.01*	0.01±0.002	0	0.01±0.001	0	2.2±0.3*	0.1±0.04*
Manifestations of cannibalism %	2.5±0.3*	0.2±0.06*	0.01±0.001	0	0	0	1.4±0.2*	0

* – $P < 0.05$ in comparison with the 3 and 4 group

Table 3. Mortality, appearance of constrictions and manifestation of cannibalism, third feeding

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo . orientalis</i>	<i>Hirudo verbana</i>	<i>Hirudo orientalis</i>
Mortality %	6.2±0.9*	0.4±0.01*	0.5±0.04	0.2±0.03	0.3±0.02*	0.1±0.01*	5.0±0.7*	0.3±0.02*
Appearance of constrictions %	5.1±0.6*	0.3±0.02*	0.2±0.01	0.1±0.02	0.1±0.01*	0.2±0.01*	3.1±0.4*	0.2±0.03*
Manifestations of cannibalism %	5.5±0.6*	0.4±0.03*	0.1±0.01	0.2±0.01	0.1±0.02*	0.1±0.01*	2.5±0.5*	0.3±0.01*

* – $P < 0.05$ in comparison with the 3 and 4 group

mortality was detected (Tab. 1). Further, until the second feeding, a small percentage of mortality of *Hirudo verbana* and *Hirudo orientalis* animals fed on human blood was observed (0.5±0.03% on average). After the second feeding, the percentage of mortality of *Hirudo verbana* in group 1 remains within 8.3±0.6%, and in group 7 within 4.0±0.3% (Tab. 2). Reappearance of constrictions in an

average of 3.1±0.4% (when fed with human and rat blood) is observed. Also, there is a change in the behavior of medical leeches, they become more aggressive and bloodthirsty, cannibalism (Figs. 1 and 2) begins to show in an average of 2.0±0.3% (Tab. 2). Similar results were obtained in *Hirudo orientalis* animals, but to a lesser extent. In group 2, the appearance of constrictions is on average



Figure 1. Manifestation of partial cannibalism: *Hirudo verbana* Carena, 1820



Figure 2. Death from human blood with traces of cannibalism: *Hirudo verbana* Carena, 1820

0.3±0.01%, mortality is 0.2±0.03% (Tab. 2). A single manifestation of cannibalism. In the 8th group, the appearance of constrictions in 0.1±0.04%, mortality in 0.2±0.01%. Cannibalism was not observed (Tab. 2). After the 3rd feeding of *Hirudo verbana*, mortality remains at the level of 6.2±0.9% in group 1, at the level of 5.0±0.7% in group 7, and the appearance of constrictions in both groups is 4.1±0.6% on average (Tab. 3). Cannibalism was observed in 4.0±0.8% of both groups. In groups 3 and 5, the mortality rate is

0.4±0.02%. Cannibalism is very rare. Similar results were obtained when feeding *Hirudo orientalis* (Tab. 3).

Discussion

As a result of the conducted research, there is a predominant mortality of animals of two species, *Hirudo verbana* and *Hirudo orientalis*, when fed with human and rat blood. During three feedings, a significant percentage of mortality (Tab. 1–3). Especially when feeding on human blood. In these groups, the appearance of constrictions and the manifestation of cannibalism is observed. Animals behave more aggressively. Some of the dead animals have bite marks (Fig. 2). When feeding blood pigs and chickens, there is a small percentage of mortality. A single manifestation of cannibalism. Cannibalism and the appearance of constrictions were observed in our previous experiments and found in some works of other scientists [14,18,19]. Reduced mortality of *Hirudo verbana* and *Hirudo orientalis* when fed with the blood of domestic and laboratory rats may indicate a thousand-year adaptation to animal blood compared to human blood. Secondly, about the reduced immunological favorability of medical leeches to human blood (an example of a transplant-host). Thirdly, in human



Figure 3. A bitten leech, which intensively secretes blood

blood, *Hirudo verbana* and *Hirudo orientalis* may contain substances that act on medical leeches in the form of addiction and attraction. As a result, they show more partial cannibalism. In order to get this blood from another animal. It should also be noted that leeches intensively spit out eaten human blood compared to others. What is possible can be, first of all, salvation from the attack of other animals. Because they are less active after feeding. Secondly, to escape from the immunological response from the blood of the host, with the volume of which it is not able to cope. It should also be noted that animal mortality decreases with each feeding. Maybe the animals are adapting to the blood they eat. In the future, this phenomenon will be analyzed. Now, according to the received data, we see that the blood of pigs, especially chickens, is the most favorable for their survival after feeding. Compared with human and non-linear laboratory rat blood.

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