Т. 9. №5. 2023
https://doi.org/10.33619/2414-2948/90

UDC 598.13
AGRIS L20
https://doi.org/10.33619/2414-2948/90/07

GENTLE BLOOD SAMPLING FROM TORTOISES (TESTUDINES) TO DETECT BLOOD PARASITES

©Gurbanova T., *ORCID: 0000-0002-5923-5600, Ph.D.,*
Institute of Zoology, Ministry of Science and Education Republic of Azerbaijan,
Baku, Azerbaijan, turkan.qurbanova@gmail.com

ЩАДЯЩИЙ МЕТОД ВЗЯТИЯ ПРОБ КРОВИ У ЧЕРЕПАХ (TESTUDINES) С ЦЕЛЬЮ ВЫЯВЛЕНИЯ КРОВЕПАРАЗИТОВ

©Гурбанова Т. Ф., *ORCID: 0000-0002-5923-5600, канд. биол. наук,*
Институт зоологии Министерства науки и образования Азербайджанской Республики,
г. Баку, Азербайджан, turkan.qurbanova@gmail.com

Abstract. Blood can be drawn from the jugular, brachial, femoral, ventral and dorsal coccygeal veins, occipital venous sinus, and heart from most species of tortoises and aquatic turtles. An alternative method for obtaining high-quality blood samples from tortoises of the genus *Testudo* for the detection of blood parasites by blood extraction using a medicinal leech (*Hirudo medicinalis*) has been proposed. One or two leeches were placed on each individual, depending on the body weight of the turtle. The time spent by leeches on blood sucking took from 20 minutes to 1 hour (average 35 minutes). After the pumped leeches fell off, they were immediately placed in sterile Petri dishes, where they contacted with a cotton swab moistened with ethyl alcohol in order to cause regurgitation of the sucked blood. In a comparative study of microscopic specimens prepared from blood samples of the spur-thighed tortoise obtained by two methods of hemorrhage: cardiopuncture and medical leeches, no qualitative differences were found. In all specimens, blood cells and intraerythrocytic stages of blood parasites were equally suitable for identification. This method of blood drawing is also effective, as well as traditional methods of blood sampling from turtles, but in contrast to them, it is simple to perform, more humane and safer for these animals.

Аннотация. Кровь у большинства видов сухопутных и водных черепах можно извлекать из яремной, плечевых, бедренных, вентральной и дорсальной копчиковых вен, затылочного венозного синуса и из сердца. В этой статье предложен альтернативный метод получения качественных проб крови у черепах рода *Testudo* на предмет выявления у них кровепаразитов путем кровоизвлечения посредством медицинской пиявки (*Hirudo medicinalis*). На каждую особь ставились по одной или по две пиявки в зависимости от массы тела черепахи. Время, затрачиваемое пиявками на кровоизвлечение составляло от 20 минут до 1 часа (в среднем 35 минут). После отпадания насосавшихся пиявок они тотчас помещались в стерильные чашки Петри где на них контактно воздействовали ватным тампоном смоченным этиловым спиртом для того чтобы вызвать срыгивание извлеченной крови. При сравнительном исследовании микроскопических препаратов приготовленных из проб крови средиземноморской черепахи полученных двумя методами кровоизвлечения: кардиопункцией и с помощью медицинских пиявок, не было обнаружено качественных различий. Во всех препаратах форменные элементы крови и внутриэритроцитные стадии кровепаразитов были одинаково пригодны для идентификации. Данный метод кровоизвлечения также результативен, как и традиционные способы взятия крови у черепах, но в отличие от них прост в исполнении,

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более гуманен и безопасен для этих животных.

Keywords: blood sampling, *Hirudo medicinalis*.

Ключевые слова: взятие проб крови, медицинская пиявка.

The development of methods for drawing blood from reptiles and turtles in particular for the detection of blood parasites is of great scientific and applied importance [1, 2]. But in most cases, obtaining blood safely and without causing suffering to the animal is often a big problem. The problem of hemorrhage is especially acute in rare and endangered representatives of the herpetofauna, such as turtles of the genus Testudo [3].

Blood can be drawn from the jugular, brachial, femoral, ventral and dorsal coccygeal veins, occipital venous sinus, and heart from most species of tortoises and aquatic turtles [4]. Choosing an appropriate method in all cases often depends on the species of turtle (the size and biological characteristics, etc.). The amount of blood that can be withdraw varies depending on the number of planned clinical and laboratory studies, but the maximum amount of blood drawn is 0.6% of body weight (6 ml per kg) [4].

One of the traditional methods for blood drawing in reptiles is cardiopuncture. With all its advantages, this method, which allows obtaining adequate blood samples, is dangerous, requires high precision and, with the slightest technical deviation from it, can either cause contamination of blood samples with fluids of the chest and pleural cavities, or the death of the animal. In some cases, this procedure can even lead to cardiac tamponade [1].

The method for drawing blood by declawing, amputation or tip excision of tail are also widely used. By amputation of the tail, it is possible to obtain the amount of blood required for most studies, but the results are not always encouraging. For example, activation of blood coagulation can distort the results obtained with this procedure. In addition, blood samples are contaminated with tissue fluids and debris [5].

A more adequate method is to puncture the tail vein located on the ventral part of the coccygeal vertebra. However, this method is quite time-consuming and difficult to perform and does not always allow obtaining a sufficient amount of blood for research. All of the listed methods of hemorrhage cannot be considered completely humane and ethical (https://goo.su/rv0ZqXH), they are often difficult to perform, as well as not always suitable for conducting research on a large amount of material [3]. Therefore, there is still a need to find alternative, more humane and technically simple methods for drawing blood from reptiles. The aim of our study was to establish the possibility of obtaining high-quality blood samples from turtles (in order to detect blood parasites) by blood drawing using a medicinal leech (*Hirudo medicinalis* Linnaeus, 1758) as the simplest and at the same time humane and safe method for these animals.

Material and methods

Two groups of spur-thighed tortoises (*Testudo graeca*) delivered to the laboratory of Protozoology of the Institute of Zoology served as the material for the study to identify blood parasites: 1) individuals caught from different natural ecosystems; 2) individuals hit by vehicles on highways, with significant damage incompatible with life.

In individuals of the first group of turtles, blood sampling was carried out by placing medical leeches on a relatively thin area of skin in the region of the base of the hind limbs and tail. Leeches were purchased through a pharmacy chain. The skin of the turtles in the place where the leeches

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were placed was carefully treated with water before the procedure. One or two leeches were placed on each individual, depending on the body weight of the turtle. The time spent by leeches on blood sucking took from 20 minutes to 1 hour (average 35 minutes). After the pumped leeches fell off, they were immediately placed in sterile Petri dishes, where they contacted with a cotton swab moistened with ethyl alcohol in order to cause regurgitation of the sucked blood. The amount of blood received from a leech did not exceed 1.5 ml.

In individuals of the second group of turtles (delivered with serious injuries), hemorrhage was performed by the standard method of cardiopuncture [4].

From the blood samples obtained by the above methods, thin smears were prepared on slides, part of the blood was also collected in Eppendorf tubes with the addition of 96% ethanol for further molecular studies. The prepared smears were dried, fixed in absolute methanol for 5 minutes and stained according to Romanovsky-Giemsa [6]. The prepared smears were examined under a Leica DM 1000 light-optical microscope with a ×100 objective and photographed with a Leica DFC 425 digital camera.

Results

In a comparative study of microscopic specimens prepared from blood samples of the spur-thighed tortoise obtained by two methods of hemorrhage: cardiopuncture and medical leeches, no qualitative differences were found. In all specimens, blood cells and intraerythrocytic stages of blood parasites were equally suitable for identification (Figure).

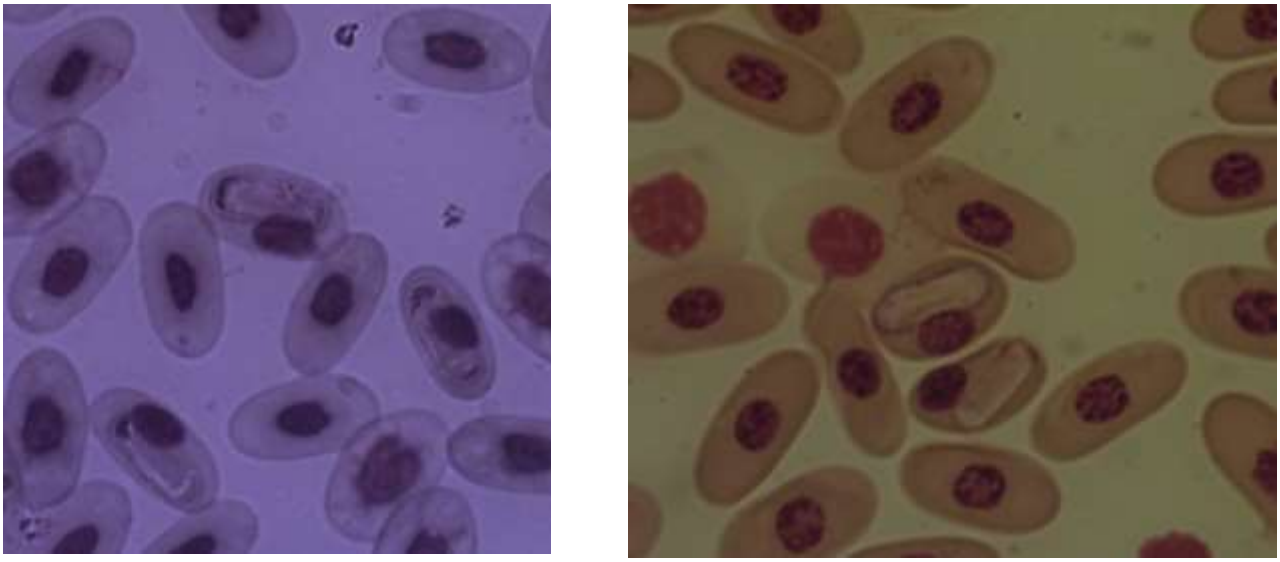

Figure. Blood parasites of the *Hemolivia* genus in erythrocytes of the spur-thighed tortoises. In the photo on the left — a specimen prepared from blood samples taken by cardiopuncture, on the right — a specimen prepared from blood samples extracted using medical leeches

Conclusion

A comparative study of two methods of hemorrhaging for obtaining high-quality blood samples from the spur-thighed tortoise (*Testudo graeca*) in order to detect blood parasites in them showed that the method of blood drawing using a medicinal leech (*Hirudo medicinalis*), as effective as the traditional method of hemorrhage, but unlike the latter, it is simpler to apply, more humane and safer for these animals.

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Работа поступила
в редакцию 06.04.2023 г.

Принята к публикации
11.04.2023 г.

Ссылка для цитирования:

Gurbanova T. Gentle Blood Sampling From Tortoises (Testudines) to Detect Blood Parasites // Бюллетень науки и практики. 2023. Т. 9. №5. С. 64-67. https://doi.org/10.33619/2414-2948/90/07

Cite as (APA):

Gurbanova, T. (2023). Gentle Blood Sampling From Tortoises (Testudines) to Detect Blood Parasites. *Bulletin of Science and Practice, 9*(5), 64-67. https://doi.org/10.33619/2414-2948/90/07