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DIAGNOSTICS OF HEMOPARASITES OF AMPHIBIANS

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Abstract. *This article presents the results of complex clinical, laboratory examination of a series of clinical cases of haemoparasites spread in the experimental and control group of amphibians of the pond frog species *Pelophylax lessonae*, caught on 7.05.2022. On the basis of the obtained results, the dependence of haemoparasites spread on conditions and period of keeping was revealed. The judgment that the longer the group of individuals is in artificial keeping conditions, the higher it has indicators on extensiveness, an average intensity of an invasion and an index of abundance of parasites.*

Keywords: haemogregarins, haemoparasites, amphibians, *Hepatozoon magna*, *Pelophylax lessonae*

In the trend of terrariumistics, it is common to trap many species of reptiles and amphibians from the wild and then move them to man-made environments in order to breed, sell or add to the terrarium keeper's personal collection. Often the owner is not

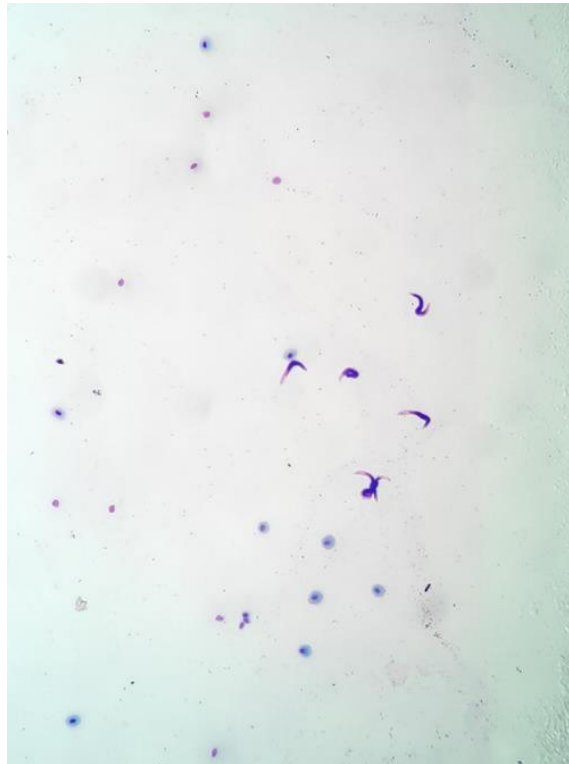
aware of the hidden dangers. For example, it has been observed that when live amphibians are fed from nature, infestation of other captive animals may occur due to invasive cystic stages [13]. Primarily exotic amphibians originating from the wild can be infested with various parasites, including zoonotic species such as *Armillifer armillatus pentastomata* [3, 4] and *Porocephalus* spp. [5, 6]. In general, all haemogregarins differ only in the details of their life cycle. Only Family Haemogregarina is characteristic of aquatic reptile species [7,8,10]. For example, Haemogregarina of the genus *Schellackia* is common in many frog species [11]. All haemoparasites have a similar intra-erythrocytic shape. The problematics of diagnostics of reptile and amphibian blood parasites lies in the fact that not all literature sources have up-to-date data on the description, distribution, life cycle, etiology and diagnostics of hemoparasites. In the CIS territory in 2007 for the first time were obtained data on the blood parasite fauna of tailless amphibians of Kyrgyzstan; 12 species of Anura haemoparasites were described, of which 8 species were first discovered in Central Asia. 12 species of different blood parasites were found, including: rickettsiae, sporophytes, trypanosomes and microfilariae [1].

In Russia, the results of the study of haemoparasites of the lake frog *Pelophylax ridibundus*, from water bodies of the North-Western Caucasus, which, together with the pond frog *Pelophylax lessonae*, is a member of the Green Frog genus, were described. The species composition of parasites was found to be represented by three species - *Hepatozoon magna*, *Dactylosoma ranarum* and microfilariae. Differences in the morphology of the erythrocytes of the lake frog when affected by sporophytes were determined [2].

The therapy of blood-parasitic diseases is problematic and not always necessary. Very few drugs are used for etiotropic therapy. As a rule, these are drugs of such series as Hindin and Hinacrine. These only stabilize the infestation and do not cause complete elimination of the parasite [12]. The pond frogs *Pelophylax lessonae*, a genus of the true frogs, which is widespread in central Europe, were chosen as an object of research.

The researches were carried out in the laboratory of the Veterinary Medicine Department of Russian State Agrarian University - Moscow Timiryazev Agricultural Academy. Experimental and control groups of pond frogs were formed, taking into account gender. The best methods for diagnosing haemoparasitosis are the preparation of native blood smears. Smears were performed on clean, degreased slides, using ground slides. Blood was taken from each individual of both groups. For blood staining in herpetology, the Romanowsky-Giemse staining method is used. The specimen is fixed with absolute 96% ethanol. The prepared smear was air-dried and the sequence number of the individual and the group it belonged to was inscribed on its surface, close to the beginning of the smear. For staining, 1 part of fresh Gimes dye dissolved in 10 parts of distillate with buffer added was used. The preparation itself should stain for at least 45 minutes. Intracellular parasites are usually examined in preparations stained with hematoxylin-eosin or azur-eosin universal dyes. Microscopy was then carried out at *60 and *100 magnification.

Research results. During the period of the research work (1.07.2022 to 28.07.2022), the presence of parasites in native blood smears was detected in 7 of 11 individuals of pond frogs. In the experimental group, haemoparasites were detected in 5 of 6 individuals. In the control group, haemoparasites were detected in 2 of 5 individuals. In males (n=9) parasites were found in 4 individuals, which is 50% of the total number of males, in females (n=2) parasites were found in 2 individuals, which is 100% of the total number of females.



Pic. 1 Hepatozoon magna in a blood smear of a Pond Frog

Microscopy of all smears of each individual from both groups revealed the presence of haemoparasites, which are characteristic of many frog species. The species composition of the parasites was found to be represented by one species, *Hepatozoon magna* sporozoa. This sporozoan species is represented by both free and intra-erythrocytic gamonts (Figure 1). The length of the free gamont varies from 27.6 to 36.7 μm (mean $32 \pm 2 \mu\text{m}$), the maximum width from 2.9 to 4.6 μm (mean $3.5 \pm 0.6 \mu\text{m}$), the distance from the anterior end to the nucleus margin from 7.2 to 11.5 μm (mean $9.4 \pm 1.3 \mu\text{m}$) and the nucleus length from 4.5 to 8.0 μm (mean $6.7 \pm 1 \mu\text{m}$). Free gamont is worm-shaped and usually slightly curved, with rounded anterior and posterior cell ends. The anterior end of the gamont is slightly wider than the posterior end. The nucleus is granular, without a distinct nucleus, and is located in the anterior third of the body. The nucleus extends along the longitudinal axis of the hamonte and occupies almost its entire width.

Table 1

Results of calculations of extensivities, intensivities and parasite abundance index of experimental and control groups

The group type	Intensity of infestation	Average intensity of the infestation	The parasite abundance index
The experimental	83,3%	37	30,8

group			
Control group	40%	25	10

On the basis of the study of the results obtained for the calculation of the extensiveness, invasion intensity and parasite abundance index of the experimental and control groups, it was found that the indicators of the experimental group of individuals were significantly higher than those of the control group (Table 4). Taking into account conditions of keeping and period of keeping of both groups in artificial conditions the conclusion can be made that the longer the group is kept in artificial conditions the higher its indices of invasion intensity, average invasion intensity and the index of parasite abundance are.

Conclusion. Thus, we can conclude from the obtained results that the distribution of haemoparasites in the experimental and control groups of amphibians from the general biotope is almost ubiquitous. For the best diagnosis of haemoparasites is the method of performing native blood smear and its staining by the Romanowsky-Giemsa method with further microscopy. According to the results of microscopy, it was found that the species composition of parasites is represented by one species - Hepatozoon magna sporozoites. When calculating and comparing the indices of extensiveness, average invasion intensity and parasite abundance index, the dependence of the spread of haemoparasites on the conditions and period of housing was revealed. Based on the results of the experimental and control groups, we can confirm the judgement that the longer a group of individuals is kept in artificial conditions, the higher its indices of extensiveness, average invasion intensity and the parasite abundance index are.

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СЕКЦИЯ «АКТУАЛЬНЫЕ ПРОБЛЕМЫ ОБЩЕЙ И ЧАСТНОЙ ЗООТЕХНИИ»

УДК 636.32/.38

ВЛИЯНИЕ СТИМУЛИРУЮЩИХ ПОДКОРМОК НА РЕПРОДУКТИВНЫЕ ПОКАЗАТЕЛИ ПЧЕЛИНЫХ МАТОК

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***Аннотация:** В статье представлены результаты исследований яйценоскости маток при использовании стимулирующих подкормок в весенне-летний период. Проведена сравнительная характеристика результатов при использовании различных типов подкормок.*

***Ключевые слова:** яйценоскость, пчеловодство, пчелиные матки, стимулирующие подкормки.*

Введение:

В весенне-летний период необходимо выполнять работы, направленные на ускорение темпов развития пчелиных семей. Доброкачественные кормовые запасы - основа содержания сильных пчелиных семей. При уменьшении их в гнезде необходимо восполнять недостаток особенно в весенний период, так как при обильном запасе корма матка откладывает большее количество яиц, семья быстрее и лучше развивается весной и наращивает большую силу к главному медосбору. Яйценоскость маток является важнейшим показателем, который на прямую характеризует потенциальную силу семьи при подготовке к продуктивному медосбору.