

Conference Paper

Studying Cell-like and Humoral Factors of Immunity and the Definition of Immune Status in Sheltopusik Lizards (*Pseudopus apodus*) with the Helminthiasis Caused By *Entomelas Sp.* Nematodes

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Abstract

Sheltopusik lizards (*Pseudopus apodus*) are natural carriers of the nematode *Entomelas* sp., which are capable of striking the majority of lizard species, quite often causing their deaths. In this article, the parameters of the immune status of the sheltopusik lizard with helminthiasis are described, as are the changes when deworming is performed with the medicine "Reptilife-plus" and immunocorrections with the medicine Immunofan.

Keywords: comparative immunology, lizards, veterinary herpetology, helminthiasis.

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Received: 23 January 2018

Accepted: 20 April 2018

Published: 3 May 2018

 Publishing services provided by
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Selection and Peer-review under the responsibility of the Amphibian and Reptiles Anomalies and Pathology Conference Committee.

1. Introduction

Scientists have established that reptiles are capable of having both a cell-like and humoral immune response. Humoral factors (lysozyme, a system of complementary interferon-like factors and transferrins), cell factors (englobing cells - heterophils and other granulocytes, monocytes and macrophages) and NK-cells are congenital factors of reptile immunity. T- and B-like lymphocytes and various classes of immunoglobulins are factors in the adaptive immunity of reptiles: M, Y, at some X [1, 2, 11, 12]. A decrease in immunologic indexes upon the use of anthelmintics containing ivermektins and avermektins was shown in mammals (rabbits, mice and calfs) [9]. An increase in immunologic indexes upon the application of immunomodulators was shown in calfs [10]. The parameters of the immune status of the sheltopusik lizard are determined for the first time in this research. The treatment of a nematodosis of the sheltopusik lizard with the medicine "Reptilife-plus" and immunocorrection with the medicine Immunofan were carried out for the first time.


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2. Methods

The objects of the research were 4 male sheltopusik lizards (*Pseudopus apodus*) weighing 300 to 340 g. All the animals were caught in Adler in the territory of Sochi (hilly terrain) and were kept captive for 2 years. Blood for analysis was taken from a ventral tail vein by means of the syringe with a heparin: the amount taken was 0.6 ml for 100 g of body weight. During a leukogram conducted on the dabs of blood from the lizards, the quantity of heterophils and lymphocytes was considered [6]. To conduct research on the phagocytic activity of heterophils from lizard feces, a test culture of *Escherichia coli* bacteria was marked out. The research technique is based counting the englobing cells in the dabs of blood and defining the indices of absorption and digestion in relation to a test-microbe [3]. During microscopy, we defined 25 heterophils (the main englobing blood cells of reptiles) and the quantity of microbes captured by them. The absorbing ability of blood cells is characterized by the following indices: phagocytosis percentage and the relation of the number of heterophils which participate in the absorption of microbial bodies to the number of all the counted heterophils. A phagocytic index is the quantity of the microbes taken by one heterophil. The digesting ability was estimated by finding an index of the completeness of a phagocytosis, which was defined in the following way. During the microscopy of the dabs of blood, the numerical relation of the damaged microbial bodies to the number of those absorbed ("A" index) was found in 25 englobing leukocytes. The same relation was defined in dab prints ("B" index). The index of the completeness of a phagocytosis can be expressed as the difference between indices "B" and "A". The rate of an index of the completeness of a phagocytosis to 1 is closer: the phagocytic activity is estimated above. Research on the common haemolytic activity of a complement of lizard blood serum was conducted: this involved preparing an isotonic veronal buffer, Olsver's preservative, sensibilized erythrocytes, and connecting erythrocytes with serums. Haemolytic activity was estimated by calculating the not-split erythrocytes in Goryaev's camera. The calculation of results was carried out according to the formula: $X = ((B-a) / B) \times 100$, where X is the common haemolytic activity of a complement; a – the number of erythrocytes in the experiment test tubes; and B – the number of erythrocytes in the control test tubes. The result was expressed in lytic units (4). When determining the number of T- and B- lymphocytes, the selection of the lymphocytic fraction in accordance with a gradient of fikoll - verografinum and environment 199 was carried out. Determining the quantity of T- lymphocytes was carried out by means of E-socket formation: this involved preparing 0,5% of a suspension of erythrocytes of a ram and connecting them

with lymphocytes with application of a glutaraldehyde.. Determination of the quantity of B-lymphocytes was carried out by the formation of an EAC- socket, which included the preparation of an EAC-complex (the erythrocytes of a ram, rabbit haemolytic serum and a complement) and its connection with lymphocytes through the application of a glutaraldehyde. T- and B-cells were counted out with a microscope, with dabs fixed according to the method given by Romanovsky - Gimza [3]. The electrophoresis of lizard serum proteins was carried in order to assess the gamma-globulin fractions of lizard blood serum in agarous gel (Cormay gel protein 100). 10 mkl of serum in gel was subjected to electrophoresis for 20 minutes at 100 B, painted: it was then dried and scanned in a densitometer at 600 nanometers. The relative and absolute (g/dl) size of the proteinaceous fractions was determined on the basis of data on the level of the common protein [7]. The complex injection antiworm medicine "Reptilife-plus" (developed by the department of herpetology at Moscow Zoo and the scientific center Agrovetzashchita) was applied to treat nematodosis in all the lizards. Prasiquantel, Dexamethazonum and Ivermectin are parts of this medicine. The drug was injected in a intramuscularly single-passly at a dose of 0.4 ml/kg [5]. The medicine "Immunofan" was applied 5 days later to produce an intramusclary immunocorrection at a dose of 0,1 ml/kg: it was then applied every other day for 2 weeks.

3. Results

To diagnose helminthiasis, the feces and an aspirate from the trachea where larvae of pulmonary nematodes *Entomelas sp.* are found, was conducted from the *Rhabdiasidae* family. The puberal forms of these helminths live in wild lizards, causing exudative inflammation [5, 8]. In the table, it is visible that a heterophil profile of the blood was revealed in most of the animals. After applying an anthelmintic, the quantity of lymphocytes decreased by 59.5% in comparison with the tentative level, while the heterophil quantity increased by 29.3%. After the application of Immunofan, the quantity of lymphocytes increased by 36%, while the heterophils decreased by 12%. In the further description, we provide a comparison of the results on reptiles with references to mammals.

The anthelmintic medicine "Reptilife-plus" had an antiparasitic effect on *Entomelas sp.* and the expressed immunosuppressor action on reptiles (tab. 1), causing a falloff in the indices for the phagocytic activity of heterophils. The phagocytosis percentage was (PP) 37.5% (in rabbits – 12.8%), the phagocytic index (PI) was 44% (in rabbits – 2%), the index of the completeness of a phagocytosis (ICP) was 94.5% (in rabbits

TABLE 1: The Hematological and immunologic indexes of the blood of sheltopusik lizards (*Pseudopus apodus*) during infection with nematodes (before treatment), after the use of the medicine "Reptilife-plus" (after treatment) and after the use of the medicine "Immunofan" (after immunostimulation).

Indices	Units of measure	Before treatment	After treatment	After immune stimulation
Quantity of lymphocytes	% in a leukocytic formula	42±7	25±5	34±3
Quantity of heterophils	% in a leukocytic formula	58±7	75±5	66±3
Phagocytosis percent	%	91±2	67±6	85±2
Phagocytic index	-	16.8±2	9.3±0.8	14.2±1.6
Index of completeness of a phagocytosis	-	0.22±0.05	0.04±0.01	0.05±0.01
Quantity of B-lymphocytes	% of all lymphocytes	21±3	13±1	20±3
Quantity of T-lymphocytes	% of all lymphocytes	18±3	13±2	21±2
Activity of a complement	Lytic units	70.1±1.3	24.2±2.2	35.2±2.8
Quantity of gamma globulins	% of all proteins of serum	3.3±0.5	0.8±0.1	2.0±0.4

- 51%), the haemolytic activity of a complement was 69%, the number of T - and B-lymphocytes was 33% (in rabbits -20%), quantity of gamma-globulins was 75% (in rabbits - 8%), the increase in the quantity of heterophils was 29% (in rabbits the quantity of neutrophils increased by 16%) and the decrease in the quantity of lymphocytes was 40.5% (in rabbits - 7.7%).

The "Immunofan" immunomodulator activates cell-like and humoral factors in the congenital immunity of reptiles. The indices of the phagocytic activity of heterophils were as follows. The phagocytosis percentage (PP) was 10% (in calfs - 43%), the phagocytic index (PI) 30% (in calfs - 90%), the index of the completeness of a phagocytosis (ICP) was 50%, the haemolytic activity of a complement was 60%, the quantity of lymphocytes increased by 36% (in calfs - 16%) and the quantity of heterophils decreased by 22% (in calfs - 12%). Immunofan caused a sharp increase in the indices of adaptive immunity: the number of T - and B-lymphocytes increased by 50% (in calfs - 56%) and the gamma-globulins by 30% (in calfs - 36%).

4. Conclusion

1. The multifold clinical inspection of the lizard *Pseudopus apodus* (sheltopusik) showed that captivity for 2 years led to an aggravation of the pulmonary nematodosis caused by *Entomelas* sp nematodes.
 2. The anthelmintic medicine "Reptilife-plus" had an antiparasitic effect on *Entomelas* sp. and the expressed immunosuppressor action on reptiles.
 3. The "Immunofan" immunomodulator activates the cell-like and humoral factors of reptile congenital immunity and causes a sharp increase in the indices of adaptive immunity.
 4. Within this research, the applied immunomodulator did not allow us to restore activity to the majority of factors in the immune protection of the lizard *Pseudopus apodus* to a datum level. This suggests the need to work off the courses of treatment.
1. The approbation of the methods for assessing the immune status accepted in the veterinary medicine of mammals showed that the defining methods (the phagocytic activity of heterophils, the assessment of the haemolytic activity of a complement, the quantitative definition of B-lymphocytes in blood) can be applied to research on the immune status of reptiles. However, the method for determining the quantity of T-lymphocytes demands further optimization for reptiles.

Acknowledgements

I express gratitude to the candidate of veterinary sciences D. B Vassilyev, the leading herpetologist of Moscow Zoo, for his help in diagnosing the disease, granting medicines for treatment, and useful recommendations about veterinary herpetology.

References

- [1] Kollias GV: Immunologic aspects of infectious diseases. In: Hoff GL, Frye FL, Jacobson ER: Diseases of amphibians and reptiles. London, New York: Plenum Press; 1984: 660 – 691.
- [2] Origi FC: Reptile immunology. In: Jacobson ER: Infectious diseases and pathology of reptiles. New York: CRC Press, Taylor & Francis group; 2007: 131 - 166.

- [3] Cooper EL: Comparative immunology. Englewood Cliffs, Prentice –Hall, Inc. New Jersey; 1976: 380 p.
- [4] Zhelankin RV, Pavlov GV: Immune and haemopoetic system of reptiles. Moscow: The research centre of quality of preparation of experts of the Ministry of Education of RF publishing; 2008 [in Russian].
- [5] Zemskov AM: Clinical immunology. Moscow: GEOTAR-Media; 2005 [in Russian].
- [6] Yemelyanenko PA: Methodical instructions on the testing of the natural resistance of calfs. Moscow: All-Union academy of agricultural sciences of the name of Lenin; 1980 [in Russian].
- [7] Vassilyev DB: Helminthosis in captive reptiles and the use of modern parasitocidal preparations in terrarial practice. Moscow: Scientific research in zoological parks; 1996; issue 5: 96 – 117 [in Russian].
- [8] Khairutdinov IZ, Pavlov AV, Sokolina FM: Comparative morphology of the blood of two species of reptiles. In: Problems of herpetology: St Petersburg: St Petersburg University publishing; 2008: 415 - 423 [in Russian].
- [9] Masopust J, Dolezhalova V: Bases of immunochemical methods of researches. Prague; 1979 [in Russian].
- [10] Sharpilo VP: Parasitic worms of reptiles in USSR fauna. Kiev: Naukova dumka; 1976 [in Russian].
- [11] Mirzaeva KM: Action of the preparation “Niacid plus” on the immunobiological parameters of animals. Moscow: Veterinarnaya medicina; 2007: № 2 – 3; 35 – 37 [in Russian].
- [12] Mamykova OI: Examination of the immunobiological status of animals after dehelminthization and the means for correction: author’s abstract for a candidate of veterinary sciences dissertation. Moscow; 1989: 1 - 20 [in Russian].