Parasitology and Helminthology

Morphological and Biochemical Investigation of the *Bothridium pithonis* Blainville,1824 (Cestoda: *Diphyllobothriidae*)

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(Presented by Academy Member Irakli Eliava)

ABSTRACT. Cestodes are the most prevalent helminths in reptiles and particularly in pythons. In case of intensive invasion cestodes can cause death in pythons in the Zoological Parks. An African Rock Python (Python sebae Gmelin, 1788) was brought to Tbilisi Zoological Park from Tanzania in 2008. A month later the animal died. In a small intestine cestoda Bothridium pithonis Blainville, 1824 (one specimen) was detected. Based on the morphological investigation and its comparison with literature data its identity with Bothridium pithonis Blainville, 1824, was proved. The length of the python was 960 mm and the width at its broadest part was 15 mm. These measurements exceed the data given in the literature. The unarmed scolex was made of two spherical parts with a medial depression between them. Each part was surrounded with semicircular muscle ridges. The maximum width of the scolex was -6 mm, height -3 mm, the width of the neck in its narrow part -1.5 mm. The tape-shaped strobila was clearly segmented with 13-14 units per 1 cm of the length. Minimal width of the segments was 9-10 mm, length - 0.5 mm. Maximal width of the segments was found in upper third of the body and equaled 15 mm and the length - to 0.9 mm. Two kinds of eggs were found: immature with 0.066 mm length and 0.044 mm width and mature - 0.13 mm in length and 0.09-0.11 mm in width. For the characteristic of the nervous system of B. pithonis the activity of enzyme cholinestherase in homogenates of the helminth was studied. In different parts of strobila the activity was different and fluctuated from 45.98 to 142.0 mkmol acetylcholine/mg protein/h.

Improved quarantine period of the reptiles and intensive parasitological monitoring are required. © 2013 Bull. Georg. Natl. Acad. Sci.

Key words: African rock python, Bothridium pithonis (Cestoda), morphology, cholinesterase.

E. Wiesenhütter [1] described tapeworm-*Bothridium pithonis* found in a midgut of the *Python reticulates* which died in the Berlin Zoological Garden. He studied the scolex histology of the cestode. The author gives the pathologoanatomic picture of the injured portion of the python intestine and mentions profound changes in the mucous membrane; however, he could not state the mentioned parasite as the cause of death in pythons. Sawada and Kugi [2] suppose that a mass invasion of reptiles by cestodes and enterohepatitis induced by endoparasites cause death in Japanese reptiles within a few days. The authors described 8 species of the



Fig. Bothridium pithonis Blainville, 1824 (Original). A) scolex, lateral view; B) scolex, apical view.

Bothridium genus including *Bothridium pithonis*. They note that the reptiles brought out of the natural habitats have already been infected with helminths. During post mortem examination of Indian Rock python (*Python molurus*) in the Madras Snake Park (India) [3] and National Zoological Garden of Sri Lanka [4] *B. pithonis* was found. Jacobson [5] mentions the pathological effect of *Bothridium* and *Bothriocephalus* on constrictor snakes.

Information about cestoda invasions in pythons in Georgia (Caucasus) is represented in the work of Kamalov [6], who studied 5 dead specimens of the reticulated pythons (*Python reticulatus*) in the Tbilisi Zoological Park. One species of cestodas found in small intestines was identified as *Salenophorus megalocephalus* (Creplin, 1839), syn.: *Bothriocephalus pythonis* (Retzias, 1829). The description of the helminth is not given in this work. It is only mentioned that the length of the longest specimen was about 50 cm.

In January 2008 African Rock Python (*Python sebae*) died in the Tbilisi Zoological Park after some months of conveyance from Tanzania. The length of the python was 3m 70cm . In the anterior part of a small intenstine one specimen of cestoda was found. We present additional data on this helminth on the basis of morphological and biochemical studies.

Materials and methods

Morphometric description is made after investigation of both fresh and fixed slides. The cestoda was fixed in 70° ethanol after rinsing for morphological study. In order to study the composition of the scolex it was cleaned by equal mixture of lactic acid and glycerin.

For biochemical study live tissues of the cestoda were homogenized in 1:20 diluted distilled water and centrifuged. The supernatant was collected and used as enzyme extract. According to the method of Willgerodt et al. [7] cholinesterase activity was determined spectrophotometrically using acetyl-choliniodide at a concentration 1×10^{-2} M as substrate. The results were expressed as mkmol acetylcholine liberated /mg protein/h. The protein content was determined by the method of Lowry et al. [8], using bovine serum albumin as standard. The obtained results were treated statistically [9].

Results and discussion

Morphological description. The color of the cestoda body is milk white. The length of the helminth about 960 mm that noticeably exceeds literature data [2,4]. Such a large size of parasite is a common environmental phenomenon and is caused by the low-intensity of the invasion (one specimen).

The scolex (Fig. A,B) is unarmed, quite thick, consisting of two spherical parts. The scolex with a medial depression, the surface is smooth. There are two deep adhesions on two elevated upper parts of the scolex. The edges of the adhesions are surrounded with well-developed, comparatively darker semicircular muscle ridges, the openings of which are directed outside. The maximum width of the scolex is 6 mm, height 3 mm, distance between the depression and the neck 2.5 mm, diameter of each spherical part 3 mm. The morphological characteristics of the scolex of the studied cestode are similar to the description of *B. pithonis* scolex given by Sawada and Kugi [2].

The neck is narrow and fairly long. The width of the neck at the narrowest side is about 1.5 mm and widens gradually.

The strobila is tape-shaped. The body is dense, strongly muscular. Opaque segmentation is sharply defined. The segments are broader than long. In one cm of the strobila 13-14 divisions are counted. The margins of the segments are serrate. Minimum width of the segments of strobila 9–10 mm, length 0.5mm; segments reach a maximum width at the anterior third of the body and are equal to 15 mm, length 0.9 mm. The average width of the strobila – about 12-13 mm, length 0.76mm.

Depressions are found along the central part of the body. The reproductive system is located in one row on the full length and is filled with eggs in different developmental stages.

Eggs are of two types. First: small size, defective, unfertilized eggs: length -0.066 mm, width -0.044mm. Second classic oval eggs, with two-layered, transparent, reddish envelope, which has a well-developed valve at one pole and a small, dark, subterminal thickening at the opposite one. Most eggs are large in size: length-0.13 mm, width -0.09-0.11 mm. Such egg-size variation in one specimen of the parasite is known in literature [10], indicating the defective structure of the ootype.

By morphological features, especially by structure and shape of the scolex the above-mentioned cestoda belongs to the order *Pseudophyllidea* Carus, 1863, family *Diphyllobothriidae* Lühe,1910, genus *Bothridium* Blainville,1824, *Bothridium pithonis* Blainville,1824.

Biochemical data. Together with morphometric description, biochemical investigation of the species was also carried out. The enzyme cholinesterase,

which represents an important index of the nervous system of the helminths, was studied.

For the first time in cestoda Bothridium pithonis activity of cholinesterase was found, which was equal on average to 96.3±11.12 mkmol of acetylcholine/ mg protein/h (n=8). This level probably determines realization of the main function of the enzyme during nervous-muscular transmission and represents the index of the total movement activity of the tapeworm. We have found differentiations in enzyme activity in different fragments of the helminth. Activity fluctuated from 45.98 to 142.0 mkmol of acetylcholine/mg protein/h. The obtained data show unequal distribution of the enzyme in strobila, which is proved by literature data for other species of cestodes, particularly for Raillietina tetragona from the chicken [11], Bothriocephalus opsariichtydis from fishes [12] and Ophiotaenia europaea from Natrix tesselata [13]. According to the above-mentioned data, the cholinesterase, found in B. pithonis, indicates normally formed nervous system and reflects the general regularity of cestoda cholinesterase.

Conclusion

The cestoda *B. pithonis* could be one of the reasons of *P. sebae* death at the Tbilisi Zoo. At the same time, we agree with the reviews of the forequoted authors [1,2] that reptiles being brought from the natural habitats to the Zoos have already been infected with parasites that cause their death or clear the way for secondary infections. Improved quarantine period of the reptiles and intensive parasitological monitoring are required.

Suppression of the activity of the enzyme cholinesterase during the screening of antihelminth drugs, may be important for the prevention and healing of snakes living in captivity.

The cestoda is preserved in the collection of the Institute of Zoology of Ilia State University of Tbilisi.

Acknowledgement. The authors express their gratitude to the veterinary service of Tbilisi Zoological Park for giving us access to the cestoda. პარაზიტოლოგია და ჰელმინთოლოგია

ცესტოდა *Bothridium pithonis* Blainville,1824 (Cestoda: *Diphyllobothriidae)-*ის მორფოლოგიური და ბიოქიმიური შესწავლა

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2008 წელს, თბილისის ზოოპარკში ტანზანიიდან შემოყვანილ იქნა აფრიკული კლდის პითონი (Python sebae Gmelin, 1788), რომელიც ერთი თვის შემდეგ დაიღუპა. მის წვრილ ნაწლავში ადმოჩენილი იქნა ცესტოდა (1 ეგზ.). Bothridium pithonis Blainville, 1824. ცესტოდის სიგრძეა 960 მმ, მაქსიმალური სიგანე-15 მმ, რაც მნიშვნელოვნად აღემატება აღნიშნული სახეობის განაზომებს ლიტერატურული მონაცემების მიხედვით. ამის საფუძველზე ჩატარდა ახალი მორფომეტრიული აღწერა. ცესტოდის შეუიარაღებელი სკოლექსი შედგება ორი სფერული ნაწილისაგან, რომელთა შორის აღინიშნა მნიშვნელოვანი ჩაღრმავება. თითოეული ნაწილი მთავრდება ბოთრიით. სკოლექსის მაქსიმალური სიგანე აღწევს 6 მმ, სიგრძე — 3 მმ. კისრის სიგანე ყველაზე ვიწრო ადგილას არის 1.5 მმ. მკვრივი ლენტისებური სხეული მკაფიოდაა სეგმენტირებული. სიგრძის ერთი სანტიმეტრი მოიცავს 13-14 სეგმენტს. სეგმენტების მინიმალური სიგანე 9-10 მმ, სიგრძე — 0.5 მმ. მათი მაქსიმალური სიგანე მოღის სტრობილას პირველ მესამედზე და აღწევს 15 მმ, ხოლო სიგრძე — 0.9 მმ. პარაზიტის კვერცხები ორი სახისაა: მოუმწიფებელი — სიგრძე 0066 მმ, სიგანე 0.044 მმ და მომწიფებული, განვითარებული — სიგრძე 0.13 მმ, სიგანე — 0.09-0.11 მმ.

მასალის მორფოლოგიური შესწავლის საფუძველზე და ლიტერატურულ მონაცემებთან შედარებით დადგინდა ცესტოდის სახეობრივი კუთვნილება.

ცესტოდა *B. pithonis*-ის ნერვული სისტემის დახასიათებისათვის მისი სხეულის ჰომოგენატებში ბიოქიმიურად განისაზღვრა ფერმენტი ქოლინესთერაზა, რომლის აქტიფობა განსხვავებული აღმოჩნდა ცესტოდის სხეულის სხვადასხვა ფრაგმენტში და შეადგენდა 45,98-დან 142,0-მდე მკმოლ აცეტილქოლინი/მგ ცილაზე/სთ. ბუნებრივი ჰაბიტატებიდან ზოოპარკებში შემოყვანილი რეპტილიები საჭიროებენ კარანტინს და პარაზიტოლოგიურ მონიტორინგს.

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Received October, 2012