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Ecological aspects of nematodes of the family Protostrongylidae, parasites of Bovidae

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Abstract. The analysis of obtained data enabled us to record eight Protostrongylidae Leiper, 1926 species in the Bovidae of Uzbekistan, namely, *Protostrongylus rufescens, P. hobmaieri*, *Spiculocaulus leuckarti, S. austriacus, S. orloffi, S. kwongi, Muellerius capillaris* and *Cystocaulus ocreatus*. The following animals were recorded as definitive hosts: *Ovis aries, Capra hircus, C. falconeri, C. sibirica, Ovis ammon* and *O. vigneis*. The general infection rate of the animals by *Protostrongylus* reached 42.6%, *Spiculocaulus* and *Cystocaulus*, 29.8%, *Muellerius* – 15.8%. The numbers of Protostrongylidae helminths in a host reach on the average from 32 to 93 individuals. Sex ratio is 1:4 and 1:7. The number of the population depends on the age of hosts and seasons, and vary significantly. We recorded terrestrial mollusks of the following species: *Vallonia costata, Gibbulinopsis signata, Pupilla muscorum, Pseudonapaeus albiplicata, Ps. sogdiana, Bradybaena lantzi, Br. phaezona, Br. dichrizona, Leucozonella ferganica, Xeropicta candacharica, Candaharia levanderi* and *Macrochlamys sogdiana,* as intermediate hosts of the nematodes. The number of infective larvae in the feet of intermediate hosts vary on the average 1 to 40 individuals. The age structure of the population of the Protostrongylidae family consists of mature individuals, larvae of the 1st, 2nd and 3rd instars. The lungs of the Bovidae are the environment for mature nematodes, while the 1st instar larvae inhabit external environments, and the larvae of 2nd and 3rd instars, terrestrial mollusks. The structure of the population of Protostrongylidae nematodes comprises free-living and parasitic groups.

Keywords: Protostrongylidae; Bovidae; Larvae; Terrestrial mollusks; Population structure.

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Introduction

Nematodes are widespread in biogeocenoses of Uzbekistan; they are accepted as pathogens of vertebrate animals in aquatic and terrestrial ecosystems. Though nematodes are important pathogenic agents causing diseases in domestic and wild ruminants, we still have limited knowledge of their hosts, geographic distribution, ecology, biology and evolutionary traits, all these being crucial data that should be incorporated into the development of an integrated strategy for their control.

Nematodes of the family Protostrongylidae Leiper, 1926 are a monophyletic group, including 60 named species belonging to 13 genera and 6 subfamilies (Boev, 1975; Anderson, 2000; Hoberg et al., 1995; Carreno and Hoberg, 1999). They adapted to parasitizing the respiratory organs of mammalian animals, mainly the Bovidae. Across Uzbekistan, there is a diverse fauna represented by 15 species of protostrongylids in Caprinae and Antilopinae with parasites distributed among 8 species of wild animals: *Capra falconeri*, *C. sibirica*, *Ovis ammon, O. vignei*, *Gazella subgutturosa*, *Saiga tatarica*, and species of domestic hosts: *Ovis aries*, *Capra hircus* (Kulmamatov et al., 1994; Akramova, 2003; Kuchbaev et al., 2004). Assemblages of hosts and parasites inhabit deserts, semi-deserts and mountains and are an important component of the corresponding ecosystem; parasitic infection is associated with significant disease processes in wild and domestic hosts.

Terrestrial mollusks of genera *Xeropicta*, *Pseudonapaeus*, *Bradybaena*, *Macrochlamys*, *Pupilla, Leucozonella,* and *Vallonia* play an important part in distribution and transmission of protostrongilidosis. The larvae of instars L2 and L3 develop in these mollusks, intermediate hosts of these nematodes (Kulmamatov et al., 1994; Kuchbaev et al., 2000; 2003; Akramova, 2003).

A population of parasites, like any other organisms, inhabits a certain range, has certain numbers and structure and is characterized with certain birth and mortality rate both by phases of their development and by ages (Bauer and Lopukhina, 1977).

A study of the Protostrongylidae population, parasites of the Bovidae, will contribute to building models of parasite-host interrelations, expand the general perception of their interrelations and provide an opportunity to a certain degree to identify potential opportunities of the distribution of these parasites and level of the manifestation of diseases related to these nematodes.

Therefore, we have studied some questions of the population structure and ecological peculiarities of the nematodes from Protostrongylidae Leiper, 1926 family, endoparasites of Bovidae in Uzbekistan.

Material and methods

The analysis of the results of studies on the fauna and population ecology nematodes of the family Protostrongylidae, parasites of the Bovidae animals in Uzbekistan, was used as the material for this work. Studies have been conducted from 2005 to 2010. In this period, faunistic studies of animals inhabiting the biogeocenoses of the Uzbekistan were conducted.

Methods described by Boev (1975) and Anderson (1978) were used for the study of the species composition and morphology of Protostrongylidae nematodes. To identify morphological criteria of mature Protostrongylidae, we separated the head and tail ends of males and females and made preparations. The collected material was put in flasks, fixed in 70° ethanol and labeled for storing at the Laboratory of General Parasitology of the Institute of Zoology, Uzbekistan Academy of Sciences.

First instar larvae were isolated from the fecal of naturally and infected animals and studied as described by Berman and Orlov and updated by Nikolsky (1961) and Jenkins et al. (2005). We collected a total of 1020 fecal samples and microscopic examinations for faeces of domestic (sheep and goats) and wild Bovidae animals *(C. falconeri, C. sibirica, O. ammon, O. vignei, G. subgutturosa* and *S. tatarica)* for larvae. The first instar larvae isolated from faeces were identified to genus. We took into account the following morphological signs: larvae without dorsal cuticular spine at the top of tail and larvae with spine, the length and form of tail and body size of larvae. Specimens was preserved as vouchers in 70% ethanol.

Terrestrial mollusks, intermediate hosts of Protostrongylidae nematodes were collected in the open area of talus, in shrubs or grasses. The mollusks were collected early in the morning, when they showed a high activity and were therefore easier to note. By counting their numbers per one sq. m we calculated their density. The identification of terrestrial

mollusks was conducted by the method of Shileiko (1978, 1984), Likharev and Victor (1980), Pazilov and Azimov (2003).

To establish the infection rate of terrestrial mollusks (Gastropoda, Pulmonata), which are intermediate hosts of Protostrongylidae we studied 10,564 individuals of these mollusks as described by Azimov et al. (1971). This method consists in the following: 20 to 30 mollusks were placed in a Petri dish and covered with a large dish. Three to five minutes later inactive mollusks started moving and sticking to dish walls. We evoked their activity by putting the dish in the sun for 3-5 min. Macroscopically, the infected parts of the tissue of mollusk feet appeared in the form of dark spots from brown to black, measuring from 2 to 3 mm (figure 1). By examining the feet of these mollusks with a magnifier we established levels of their infection by Protostrongylidae larvae. Besides, we counted these larvae and thus established the intensity of the infection of the intermediate hosts.

Figure 1. A mollusk stuck to the Petri dish. Protostrongylidae larvae are readily seen in the foot of the mollusk

To identify the level of infection of Bovidae by Protostrongylidae nematodes, we conducted complete helminthological dissections as described by Skryabin (1928). We examined 258 lungs of 210 domestic and 48 wild Bovidae animals. Protostrongylidae nematodes were mainly recorded in the associated form (combined). The number of species in one infected sheep or goat varried from 2 to 5. The absolute infection rate and the intensity of the infection are given at the genus level. The lungs of seven wild Bovidae animals were brought from Tashkent Zoo, namely, *G. subgutturosa* – 3; *C. sibirica* – 2; *O. ammon* – 2. Forty-eight animals were brought from natural reserves: *O. ammon*, 11, from Nurata reserve; *C. falconeri*, 6, and *O. vignei*, 10, from Kugitang nature reserve; *C. sibirica*, 4 from nature reserve of Chatkal; *G. subgutturosa*, 4 from of Ecocenter of "Jayron" of the State of the Committee of Nature of Uzbekistan; *S. tatarica*, 8, from Ustyurt Plateau of Karakalpakstan.

We studied the lungs of 88 sheep and 56 goats from the Fergana Valley (farms of Namangan, Fergana and Andijan provinces) and 38 sheep and 28 goats in the south of Uzbekistan (farms of Surkhandarya and Kashkadarya provinces). To study the dynamics of the numbers of Protostrongylidae populations in definitive hosts *(O. aries)*, we examined the lungs of naturally infected 126 adult sheep and 54 lambs. To identify the species ratio in Protostrongylidae and their sex, we studied the lungs of individual adult sheep infected in natural conditions. The mature Protostrongylidae nematodes obtained from each infected lung were processed as the following: nematodes isolated from each animal were placed into separate Petri dishes under binocular microscope Olympus CK2 (Optical Co. Ltd, Japan) against the dark background. Each nematode species was placed by using a preparation needle into a separate dish for further studies. After Protostrongylidae nematodes had been identified, the males and females were counted.

Results and discussion

Bovidae of Uzbekistan, definitive hosts of the nematode family Protostrongylidae

The nematodes of the Protostrongylidae family got adapted to parasitizing in the respiratory organs of mainly the Bovidae animals.

Analyzing the data obtained, it is possible to draw a conclusion that the overwhelming majority of species and subspecies of the Bovidae animals have been infected by respective nematodes of the genera

Protostrongylus, Spiculocaulus, Muellerius and *Cystocaulus* (table 1).

The general infection rate of the animals by *Protostrongylus* reached 42.6%, *Spiculocaulus* and *Cystocaulus* 29.8%, and *Muellerius* 15.8 (table 1). These data suggest that sheep and goats appeared to be most infested by all Protostrongylides. So, sheep are infested by *Protostrongylus* by 50.7%, *Spiculocaulus*, 37.3%, *Muellerius* 15.1% and *Cystocaulus* by 30.9%. The goats were infected by 40.4; 25.0, 25.0, and 35.7%, respectively. Total infection of wild animals by *Protostrongylus, Spiculocaulus, Muellerius* and *Cystocaulus* is given in table 1.

Table 1. Infection levels of the Bovidae animals by the nematodes of the Protostrongylidae

Animal species	Number of lungs studied	Infected by genera: *Protostrongylus*		*Spiculocaulus*		*Muellerius*		*Cystocaulus*	
		Infected	%	Infected	%	Infected	%	Infected	%
Capra falconeri	6	2	33.3	2	33.3	-	-	2	33.3
Capra sibirica	6	2	33.3	2	33.3	-	-	2	33.3
Capra hircus	84	34	40.4	21	25.0	21	25.0	30	35.7
Ovis ammon	11	4	36.4	3	27.2	-	-	3	27.2
Ovis vignei	10	2	20.0	2	20.0	1	10	1	10.0
Ovis aries	126	64	50.7	47	37.3	19	15.1	39	30.9
Saiga tatarica	8	-	-	-	-	-	-	-	-
Gazella subgutturosa	7	-	-	-	-	-	-	-	-
Total:	**258**	**110**	**42.6**	**77**	**29.8**	**41**	**15.8**	**77**	**29.8**

The morphological study recorded eight mature nematodes in domestic and wild Bovidae, namely, *P. rufescens, P. hobmaieri*, *S. leuckarti, S. austriacus, S. orloffi, S. kwongi, M. capillaris* and *C. ocreatus*, which belong to four genera, 2 subfamilies and the family Protostrongylidae Leiper, 1926.

Protostrongylidae nematodes often parasitize Bovidae animals in the associated form. Table 2 sites the material on the rate of associated infection of sheep and goats. It is noteworthy that the number of Protostrongylidae nematodes in one infected sheep (goat) varied from 1 to 5 (table 2).

Table 2. Infection of sheep and goats by Protostrongylidae

Infection by:	Sheep			Goats		
	S	Infected		S	Infected	
		I	%		I	%
1 species	25	4	16,0	20	4	20,0
2 species	25	11	44,0	20	3	15,0
3 species	25	2	8,0	20	1	15,0
4 species	25	1	4,0	20	-	-
5 species	25	1	4,0	20	-	-

S – Studied; I – Infected.

Infected by Protostrongylidae 4 sheep and 4 goats by one species; 11 sheep and 3 goats by two species; 3 kinds 2 sheep and 1 goat by three species; one sheep by four or five species.

Dominant species in associations were *P. rufescens, P. hobmaieri, S. leuckarti, M. capillaris* and *C. ocreatus.*

The numbers of the population of nematodes the family Protostrongylidae (Ovis aries)

Definitive hosts are known to become infected by Protostrongylidae nematodes by swallowing infected mollusks together with grass in pastures of mountain ecosystems. The numbers of Protostrongylidae nematodes vary depending on the age and season. We monitored these parameters in naturally-infected lambs and adult sheep. We examined 126 lungs of adult sheep and 54 lungs of lambs; the infection level was 39.7% and 20.4%, respectively (table 3).

The populations of the considered nematodes, as well as the other organisms, have certain numbers, original composition and distribution in populations of definitive hosts. They are characterized by a specific seasonality. Thus,

the dependence of the number of a population on the host age is noted. So, the level of the number of considered nematodes in lambs depending on the season changed within on the average 32.2 ± 18.9 individuals, and 93.2 ± 15.1 in adult sheep (table 3).

Sex ratio in Protostrongylidae populations parasitizing definitive hosts (Ovis aries)

As it is known, the parasites of both sexes parasitize definitive hosts. Sexual dimorphism is distinctly expressed in nematodes. Sexual maturation in Protostrongylidae nematodes lasts 28-30 days. This provides for a multiple insemination of females and numerous ofspring. The examination of the lungs of naturally infected individual sheep revealed the ratio between males and females at 1:4 and 1:7 (table 4).

This peculiar ratio is likely to provide vital demands of each species for the realization of the reproductive potential.

Table 3. The dynamics of the Protostrongylidae numbers in definitive hosts

Seasons	Studied individuals	Infected		
		Infected	%	Number of parasites
		Lambs of the current year birth		
Spring	10	-	-	-
Summer	15	2	13.3	16.5 ± 9.1
Autumn	15	5	33.3	27.6 ± 5.5
Winter	14	4	28.6	53.7 ± 15.3
Total:	54	11	20.4	32.2 ± 18.9
		Adult sheep (2-3-year old)		
Spring	32	11	34.3	77.4 ± 15.3
Summer	32	10	31.2	84.4 ± 18.8
Autumn	31	15	48.3	103.4 ± 32.1
Winter	31	14	45.2	109.6 ± 26.1
Total:	126	50	39.7	93.2 ± 15.1

Table 4. Sex ratio in nematodes of genera of the family Protostrongylidae

No	Nematode species	Revealed individuals			
		Total	Males	Females	Ratio
1	*P. rufescens*	58	10	48	1:6
2	*P. hobmaieri*	65	9	56	1:7
3	*S. leuckarti*	46	11	35	1:4
4	*S. orloffi*	23	5	18	1:5
5	*S. austriacus*	27	6	21	1:4
6	*S. kwongi*	26	4	22	1:7
7	*M. capillaris*	18	3	15	1:5
8	*C. ocreatus*	49	12	37	1:4

Terrestrial mollusks, intermediate hosts of Protostrongylidae helminthes

It is generally accepted that terrestrial mollusks are one of the basic components of the terraneous ecosystems, which participate in the formation of soil biocenoses. They are widely represented in biogeocenoses of Uzbekistan and many mollusk species are intermediate hosts of parasitic organisms of vertebrate animals.

The recorded Protostrongylidae nematodes develop in terrestrial mollusks, their intermediate hosts. Of the studied 24 mollusk species, 12 were infected (table 5). The data given in table confirm significant infection levels of terrestrial mollusks by Protostrongylidae larvae in foothill and mountain landscapes of Uzbekistan. Their total infection by Protostrongylidae reached 38.4%.

Of terrestrial mollusks, the most widespread and infected were *Xeropicta candacharica*. The infection level by Protostrongylidae 57.5%. The population density in the Fergana Valley reached 50-60 individuals per sq. m; in the south of Uzbekistan, 15-20 individuals per sq. m.

As our studies have found out, in the natural conditions of Uzbekistan, the species of terrestrial mollusks, namely, *Vallonia costata, Gibbulinopsis signata, Pupilla muscorum,*

Pseudonapaeus albiplicata, Ps. sogdiana, Bradybaena lantzi, Br. phaezona, Br. dichrizona, Leucozonella ferganica, X. candacharica, Candaharia levanderi and *Macrochlamys sogdiana,* have been found infected by the Protostrongylidae larvae and are habitats for the parasitic populations of the larvae.

The number of infective Protostrongylidae changed from on the average 1.5 ± 0.8 up to 39.3 ± 14.1 individuals (table 6).

Table 5. Infection levels of terrestrial mollusks by Protostrongylidae larvae

No	Mollusk species	Mollusks studied	Infected by Protostrongylidae	
			Infected	%
1	*Vallonia costata*	203	20	9,6
2	*V. pulchella*	107	-	-
3	*Gibbulinopsis signata*	120	7	5,8
4	*Pupilla triplicata*	110	-	-
5	*P. muscorum*	210	21	10,0
6	*Pseudonapaeus albiplicata*	1781	638	35,8
7	*Ps. sogdiana*	210	44	20,9
8	*Bradybaena lantzi*	350	43	12,3
9	*B. phaezona*	300	19	6,3
10	*B. dichrozona*	215	7	3,3
11	*B. saturata*	101	-	-
12	*Lencozonella ferganica*	107	5	4,7
13	*L. caryodes*	65	-	-
14	*Angiomphalia regelaina*	101	-	-
15	*A. lentina*	95	-	-
16	*Xeropicta candacharica*	5550	3192	57,5
17	*Deroceras leave*	44	-	-
18	*D. strurangy*	63	-	-
19	*Candaharia levanderi*	78	18	23,0
20	*Macrochlamys sogdiana*	201	39	19,4
21	*M. turanica*	185	-	-
22	*Zonitoides nitidus*	67	-	-
23	*Oxyloma elegans*	79	-	-
24	*Succinea purtis*	222	-	-
	Total:	**10564**	**4053**	**38,4**

Table 6. The number of the population of infective Protostrongylidae larvae

Mollusk species	The numbers of L3 recorded
Vallonia costata	2,8 ± 0,8
Gibbulinopsis signata	1,5 ± 0,8
Pupilla muscorum	4,5 ± 0,6
Pseudonapaeus albiplicata	24,2 ± 3,1
Pseudonapaeus sogdiana	8,2 ± 0,8
Bradybaena lantzi	22,6 ± 5,9
Bradybaena phaezona	7,4 ± 1,1
Bradybaena dichrozona	2,4 ± 0,5
Leucozonella ferganica	17 ± 3,5
Xeropicta candacharica	39,3 ± 14,1
Candaharia levanderi	5,6 ± 1,1
Macrochlamys sogdiana	11,2 ± 1,6

In general, the infection of mollusks with the larvae of Protostrongylidae nematodes in the region under study is rather high. In the period from May to October the number of the mollusk population in pastures reaches 50-60 individuals per sq. m. In this period, the ecological situation in biotopes, as the studies suggest, contributes to the activity of mollusks and nematode larvae, which promotes their contact. The aggregate of abiotic and biotic factors contributes to the mass infection of mollusks with the 1st instar larvae, which develop to the infective stage. Mollusks infected with 3rd stage larvae actively move to the top of plants and are subsequently swallowed by definitive hosts together with the grass.

The population structure of Protostrongylidae nematodes

Our findings enable us to state that the age composition of the Protostrongylidae family consists of mature forms of males and females, larvae of the 1st, 2nd and 3rd instars. The lungs of Bovidae are the environment for mature individuals; external environments for the 1st instar larvae; and terrestrial mollusks for 2nd and 3rd instar larvae. Therefore, the structure of the population of Protostrongylidae nematodes comprises free-living and parasitic groups (table 7).

Table 7. Age structure of Protostrongylidae nematodes

Stage	State of population	Location in hosts		E
		D	I	
Adult	Parasitic	+	-	-
1st instar larvae	Free-living	+	-	+
2nd instar larvae	Parasitic	-	+	-
3rd instar larvae	Parasitic	-	+	-

D – Definitive; I – Intermediate;
E – Environment.

Taking into consideration the fact that the considered nematodes have a complex life cycle, it is logical to distribute the factors of the regulation of the numbers of their population between the factors working equally effectively throughout the whole range of the density of a population and factors manifesting more and more rigidly with the increase (Kennedy, 1978) in the population numbers at a certain phase of the development of the infective stage.

These two types of factors are not mutually exclusive and can affect this population simultaneously. Particularly, factors of the second type can lead to occurrence of a stable system from the point of view of the general theory of system. The basis of this theory is a universal model of system having an input and an output (figure 2). This model can be updated and detailed; besides, any system, when necessary, can be divided into smaller systems.

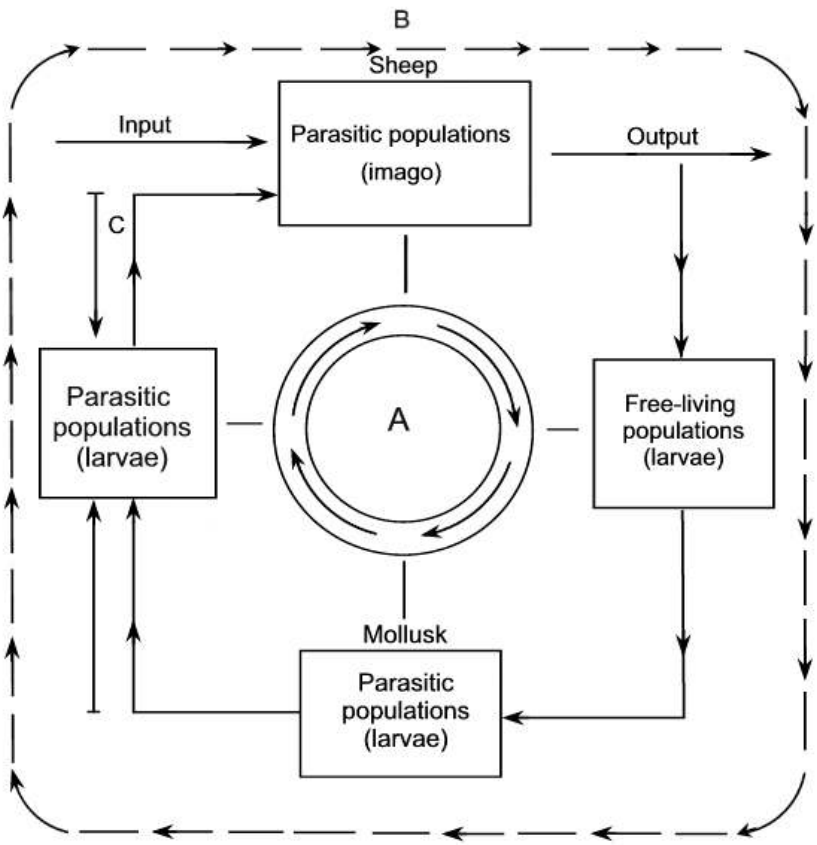

Figure 2. A model of the Protostrongylidae populations:
A – biocenotic interrelations of the parasite populations; B – parasite-host system; C – feedback from output

It is necessary to note that systems of regulation in a specific event are based on the principle of a negative feedback. Here, infective larvae penetrating definitive hosts are accepted as an input, while number of eggs (larvae), oviposited by adult individuals, as an output. To provide the balance of a population, the input should depend on the output. However, environmental factors of the exogenous character, as well as different barriers of intermediate and definitive hosts, i.e. factors of endogenous character, exercise influence on these populations. Therefore, a negative feedback enables the emergence of stable systems as a regulating mechanism.

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