

BIOLOGICAL PECULIARITIES OF SOME CATTLE NEMATODE SPECIES IN UZBEKISTAN DEVELOPING WITH THE PARTICIPATION OF DIPTHERS

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Annotation. This article presents the results of many years of our own research and literature data on the biological characteristics of some types of nematode parasites of cattle, developing with the participation of Diptera insects in the biocenoses of Uzbekistan.

In cattle of Uzbekistan, 71 species of helminths are registered, of which 12 species belong to the class Cestoda Rudolphi, 12 species to the class Trematoda Rudolphi, 1808 and 47 species to the class Nematoda Rudolphi, 1808. their adaptation to a variety of environmental conditions, contributing to the development of parasites in all phases of life cycles. Of the identified 47 species of cattle nematodes in Uzbekistan, 10 species develop with the participation of dipterous insects.

Biological features are highlighted - *Onchocerca lienalis* (Stiles, 1892), *Stephanofilaria stilesi* Chitwood, 1934, *Stephanofilaria assamensis* Pande, 1936, *Thelazia rhodesi* (Desmaresi, 1827), *Parabronema skrjabini* (Rassowska, 19240) and *Setaria labiatopapillosa* (Alessandrini, 1838) - bovine parasites livestock developing with the participation of dipterous insects in various biocenoses of Uzbekistan.

The data obtained by us will serve as a scientific basis for planning and carrying out therapeutic and prophylactic measures against pathogens of nematodosis in cattle and other ruminants that develop with the participation of Diptera in specific biocenoses of Uzbekistan.

Key words: Fauna, helminth, nematode, morphology, intermediate hosts, definitive hosts, circulation, life cycles, Diptera, invasive larva.

Introduction. One of the central problems of general parasitology is still the problem of biology and life cycles of animal parasites. It is known that the study of the life cycles of parasites is of great theoretical and practical interest; detailed knowledge of the biology of helminths makes it possible to intervene in the course of their development, breaking the most vulnerable links. Effective prevention of helminthiasis or an effective fight against them can only be organized on the basis of deep knowledge of the circulation of pathogens in nature and their development at all stages of life cycles. However, despite this, the life cycles of the vast majority of helminths remain unknown or studied extremely superficially [1].

The degree of distribution of the nematode in cattle in Uzbekistan is directly dependent on the climatic and geographical conditions of pasture areas and external factors (temperature,

humidity, etc.) that inhibit or contribute to the development and preservation of invasive elements in the environment.

Animals become infected with helminths, including nematodes, mainly on pastures, the ecological conditions of which contribute to the emergence and circulation of pathogens of invasion.

In order to develop scientifically based and practically acceptable measures for pasture prevention of nematodes in ungulates, including cattle, it is necessary to study the structure of the nematode fauna and regional features of the biology of widespread pathogens of nematodes, to establish obligate intermediate hosts of pathogens of invasions, to identify ways of transmission of invasions by intermediate hosts to the final and others

Consequently, the extraordinary diversity of the natural and climatic conditions of Uzbekistan, of course, leaves its mark on the formation and distribution of the helminth fauna, including cattle nematodes, as well as on their circulation in the biogeocenoses of this region.

An important place in the comprehensive study of helminths, including nematodes of cattle in Uzbekistan in recent years, has been occupied by studies of various invertebrates as intermediate hosts of parasitic worms.

Despite numerous studies and publications on the fauna of helminths and the biological characteristics of some species of nematodes of cattle in Uzbekistan, developing with the participation of insects (a significant part of which was made by the author) and numerous data relating to these parasites, the need to generalize and analyze the available information is obvious. [2,3,4,5,6,7,8,9,10,11,12,13,14,15].

In this context, the study of the biological characteristics of some species of cattle nematodes that develop with the participation of Diptera insects in the conditions of Uzbekistan (*Onchocerca lienalis* (Stiles, 1892), *Stephanofilaria assamensis* Pande, 1936, *Stephanofilaria stilesi* Chitwood, 1934, *Thelazia rhodesi* (Desmaresi, 1827) , *Parabronema skrjabini* (Rassowska, 19240) and *Setaria labiatopapillosa* (Alessandrini, 1838) are of certain scientific and practical interest.

Purpose of the study. The purpose of this study is to summarize and analyze the results of our long-term studies and literature data on the study of some issues of the biology of the dominant species of cattle nematodes that develop with the participation of Diptera insects in the conditions of Uzbekistan.

Materials and methods of research. The present work is based on the materials of field and experimental studies conducted over many years (1978-2019) in the laboratory of parasitology of the Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan and at the Department of Zoology and Anatomy of the Tashkent State Pedagogical University named after Nizami.

The degree of infection of cattle with nematodes was established by complete and incomplete helminthological dissections of animals, as well as their individual organs, according to the method of K.I. Scriabin [16]. 496 heads of cattle were examined by the method of complete helminthological dissections. In addition, to study the epizootology of pathogens of the main

nematodes developing with the participation of insects, the method of complete and incomplete helminthological dissections also examined about 9865 sets of individual organs of cattle at meat processing plants and slaughter sites of various livestock farms of the republic.

To detect sexually mature stephanophilaria and their larvae, after hair removal, deep scrapings of the affected skin areas (350 samples) were made from cattle according to known methods [17,18].

Helminthological material from cattle was collected in five regions of Uzbekistan: North-Eastern (Tashkent, Syrdarya, Jizzakh regions), Eastern (Fergana, Andijan, Namangan), Central (Samarkand, Navoi, Bukhara regions), (South (Kashkadarya, Surkhandarya , region), North-Western (Khorezm region, Republic of Karakalpakstan). At the same time, animals were examined at meat processing plants and slaughterhouses in Andijan, Namangan, Ferghana, Samarkand, Jizzakh, Syrdarya, Tashkent, Bukhara, Navoi, Kashkadarya, Surkhandarya, Khorezm regions and the Republic of Karakalpakstan, in all seasons.

The study of morphology and identification of parasites were carried out on temporary and permanent preparations in accordance with the determinants and descriptions given in the works of domestic and foreign researchers [19,20,21].

An important place in the comprehensive study of helminths, including nematodes of cattle in Uzbekistan in recent years, has been occupied by studies of various invertebrates as intermediate hosts of parasitic worms.

In order to identify the circle of intermediate hosts of the dominant nematode species involved in the functioning of the host-parasite system in various biocenoses of Uzbekistan in places of cattle concentration, we collected and studied more than 50 thousand Diptera insects belonging to 39 species using known methods [22, 23, 24]. The dissection of insects and the determination of parasite larvae were also carried out according to the method developed by Nelson, 1959; 1960; Nelson and Pester, 1962 [25,26,27].

Insects were studied during the flight season (May-October), only in the open air, in places of concentration of animals. We used the usual entomological methods to collect insects.

The captured insects were stained with ether and placed in Petri dishes or watch glasses. Their species affiliation was determined, and then each individual was transferred to a glass slide in a drop of physiological solution, split with dissecting needles, and examined under a microscope to detect nematode larvae. The compression method was also used. For this purpose, 5-6 Diptera were placed on a glass slide, a drop of warm (20-25) saline was applied, and examined under a microscope.

At the same time, the degree of infection of insects with invasive nematode larvae of cattle was established.

Results and discussion. According to the results of our long-term studies and analysis of literature data, 71 species of helminths were registered in cattle of Uzbekistan, of which 12 species belong to the class Cestoda Rudolphi, 1808 (*Moniezia expansa*, *M. benedeni*, *M. autumnalia*, *M. alba*, *Avitellina centripunctata*, *Thysaniezia giardi*, *Taenia hvdatigena* (larvae), *Taeniarhynchus saginatus* (larvae), *Multiceps multiceps* (larvae), *M. skrjabini* (larvae), *Echinococcus granulosus*

(larvae), *Alveococcus multilocularis* (larvae), 12 species to the class Trematoda Rudolphi, 1808 (*Fasciola hepatica*, *F. gigantica*, *F. indica*, *Dicrocoelium dendriticum*, *Eurytrema pancreaticum*, *E. coelomaticum*, *Paramphistomum ichikawai*, *Calicophoron calicophorum*, *C. erschowi*, *Liorchis scotiae*, *Gastrothylax crumifera*, *Schistosoma turkestanicum*) and 47 species to the class Nematoda Rudolphi, 1808 (*Trichocephalus*, *T. skrjabini*, *T. globulosa*, *Aonchotheca bovis*, *Strongyloides papillosus*, *Chabertia ovina*, *Bunostomum trigonocephalum*, *B. phlebotomum*, *Oesophagostomum venulosum*, *O. radiatum*, *O. columbianum*, *O. asperum*, *Dictyocaulus viviparus*, *Trichostrongylus axei*, *T. colubriformis*, *T. vitrinus*, *Camelostongylus mentulatus*, *Cooperia oncophora*, *C. zurnabada*, *C. punctata*, *C. pectinata*, *Grosspiculagia occidentalis*, *Haemonchus contortus*, *H. placei*, *Marshallagia marshalli*, *M. mongolica*, *Nematodirus filicollis*, *N. abnormalis*, *N. helvetianus*, *N. oiratianus*, *N. spathiger*, *Ostertagia ostertagi*, *Skrjabinagia lyrata*, *Teladorsagia circumcincta*, *T. trifurcata*, *Skrjabinema ovis*, *Gongylonema pulchrum*, *Parabronema skrjabini*, *Onchocerca lienalis*, *O. gutturosa*, *Setaria labiatopapillosa*, *Stephanofilaria stilesi*, *S. assamensis*, *Thelazia gulosa*, *Th. rhodesi*, *Th. skrjabini*, *Th. Zetrovi*).

As can be seen from the above materials, most types of helminths belong to the class of nematodes. We are inclined to explain the species diversity of cattle nematodes in Uzbekistan by the evolutionary adaptation of nematodes to various environmental conditions, which contribute to the development of parasites in all phases of their life cycles. Of the identified 47 species of cattle nematodes in Uzbekistan, 10 species develop with the participation of dipterous insects. Below are the results of many years of our own research and literature data on the biological characteristics of the nematode: *Onchocerca lienalis* (Stiles, 1892), *Stephanofilaria stilesi* Chitwood, 1934, *Stephanofilaria assamensis* Pande, 1936, *Thelazia rhodesi* (Desmaresi, 1827), *Parabronema skrjabini* (Rassowska, 1924) and *Setaria labiatopapillosa* (Alessandrini, 1838), parasites of cattle that develop with the participation of dipterous insects in various biocenoses of Uzbekistan.

Features of biology *Onchocerca lienalis* (Stiles, 1892). Nematodes *Onchocerca lienalis* are widespread in the plain and foothill-mountain zones of Andijan, Bukhara, Samarkand, Tashkent, Surkhandarya, Kashkadarya, Khorezm regions and the Republic of Karakalpakstan. The intensity of invasion varied from 7.0 to 15.0%. The intensity of invasion is 1-5 specimens.

They parasitize in the tendons of the knee joints in the gastrolial ligament, on the cervical ligaments of cattle, and microfilariae in the skin. In the places of parasitism, inflammatory-necrotic processes are observed, and their metabolites also have a toxic effect on the host organism. Microfilariae of *onchocercius*, migrating in the skin of the definitive host, cause serious changes in its structure. These defects significantly reduce the quality of the skin. Below are the results of an experimental study of the biological development cycle of the nematode *Onchocerca lienalis* in Uzbekistan.

The material of our study was our own collection of midges (2009 - 2015) from different biotopes of the plain and foothill-mountain zones of the northeastern region of Uzbekistan (within the Tashkent and Syrdarya regions). When studying the collected midges - potential intermediate hosts, we found that they belong to 25 species and 7 genera: *Odagmia* (8 species), *Friesia* (5),

Simulium (4), Wilhelmia (3), Eusimulium (1), Obuchovia (2), Tetisimulium (2). The most numerous species were Wilhelmia mediterranea, W. veltisthevi, Odagmia ornata, O. caucasica, Friesia alajensis, Simulium flavidum. Onchocercis larvae, at different stages of development, were found only in three species of midges - Odagmia ornata, Friesia alajensis and Simulium flavidum (Table 1).

Table 1.

Infestation of midges by onchocercius larvae in the natural conditions of Uzbekistan (2009-2015.)

Types of midges	Research		
	Total copies.	Infected, %	The intensity of invasion
<i>Odagmia ornata</i>	5150	309 (6.0)	1 - 315
<i>Friesia alajensis</i>	4550	232 (5.1)	1 - 175
<i>Simulium flavidum</i>	3675	125 (3.4)	1 - 52

As shown in Table 1, these species are quite highly infested with onchocercis larvae (3.4 - 6.0%) at an intensity of 1 - 315 and 1-315, respectively. Thus, midges *Odagmia ornata*, *Friesia alajensis* and *Simulium flavidum* are registered as intermediate hosts - parasites of cattle in Uzbekistan. In this regard, it is of interest to study the dynamics of invasion of midges (*O. ornata* and *F. alajensis*) by onchocercius larvae. The dynamics of infestation of midges *Odagmia ornata* by onchocercius larvae in different seasons of the year varied within 5.4 - 19.1%, and *Friesia alajensis* - 7.6 - 15.8% (Table 2).

Infective larvae of onchocercius enter the body of the definitive host (cattle) by biting and blood-sucking infected midges of these species. Based on the way infective larvae of this nematode entered the body of the definitive host, we undertook experimental infection of two calves (gobies of the local breed). For these purposes, invasive larvae of onchocercius from midges were used.

Animals were infected on July 10, 2010 with infective larvae of the nematode by introducing them intradermally in the area of the lower peritoneum (umbilical cord). The number of introduced larvae was 150 and 250 ind. The animals were kept under conditions precluding spontaneous infection with onchocerci. Dermolarvoscopy of skin samples of infected animals was systematically performed.

Table 2.

Seasonal dynamics of invasion of midges by larvae
onchocercus (2009-2015)

Seasons	Types of midges	Researched		
		Total copies.	Infested specimen.	Infection, %
Spring (March-May)	<i>Odagmia ornata</i>	1123	65	5.8
	<i>Friesia alajensis</i>	685	52	7.6

Summer (June August)	<i>Odagmia ornata</i>	3171	605	19.1
	<i>Friesia alajensis</i>	2519	329	13.1
Autumn (September November)	<i>Odagmia ornata</i>	943	51	5.4
	<i>Friesia alajensis</i>	922	146	15.8

For the first time, larvae of onchocercius in intermediate hosts *Odagmia ornata* and *Friesia alajensis* were found in spring - in May, 5.8 and 7.6%, respectively. The maximum infestation of *O. ornata* was recorded in summer (19.1%), and that of *F. alajensis* in autumn (15.8%). From this it is obvious that the infection of animals with onchocerci occurs in spring, summer and autumn. This position is consistent with the phenological features of the considered midges in the conditions of Uzbekistan.

As our studies have shown, the most intense flight of midges is as; in the plains and in the foothill-mountain zones of Uzbekistan was recorded in May-July. In August-September, a decrease in the number of simuliids was observed. The maximum number of midges of the second generation was observed in October, after which their number began to decrease; in winter, no winged forms were noted.

The most active midges attack "prey" in the morning with the onset of dawn from 4-5 to 9-10 o'clock and in the evening from 16-17 to 20-21 o'clock. Sometimes they attack during the daytime. During the hot part of the day, midges prefer cooler places. They could be found under large stones, in bushes, on the stems and leaves of plants hanging down to the water. In rainy hot weather (air temperature over 30°C) or in windy conditions (speed 1.5-2.0 m/s and more), flight and attacks of simuliids on animals were not recorded. In addition to winged forms, in the surveyed biotopes, we also found a large number of eggs, larvae and pupae of midges.

The results of the conducted studies allow us to state that midges are an essential component of the midges complex in the plain and foothill-mountain zones of Uzbekistan. The species composition of simuliids turned out to be quite diverse, and the population size of the dominant species reaches high limits, which are involved as intermediate hosts of pathogens of onchocerciasis in animals. The study of the found larvae of onchocercius in midges *Odagmia ornata*, *Friesia alajensis* and *Simulium flavidum* showed that microfilariae enter the body of midges with blood from the skin of infested animals during blood sucking.

First, microfilariae enter the intestine, then into the abdominal cavity and migrate to the breast. This is where the further development of the larvae takes place. From elongated larvae become short (0.113-0.118x0.010-0.013 mm), acquiring a "sausage" shape. In the process of development, the size of the larvae increases, the esophagus, intestines, and nerve ring appear. There is a morphological and biological restructuring of a number of organs. The larvae molt twice and migrate from the breast of midges to the head and proboscis. The larvae found here are usually invasive. Their size is 0.452-0.650x0.018-0.028 mm (Fig. 1).

Infective larvae of onchocercius enter the body of the definitive host (cattle) by biting and blood-sucking infected midges of these species. Based on the way infective larvae of this nematode

entered the body of the definitive host, we undertook experimental infection of two calves (gobies of the local breed). For these purposes, invasive larvae of onchocercius from midges were used.

Animals were infected on July 10, 2010 with infective larvae of the nematode by introducing them intradermally in the area of the lower peritoneum (umbilical cord). The number of introduced larvae was 150 and 250 ind. The animals were kept under conditions precluding spontaneous infection with onchocerci. Dermolarvoscopy of skin samples of infected animals was systematically performed. At the same time, *Onchocerca lienalis* microfilariae were observed 220-245 days after the animals were inoculated. In the study by the method of complete helminthological autopsies of slaughtered animals, mature nematodes *Onchocerca lienalis* were found in both calves on the cervical ligaments.

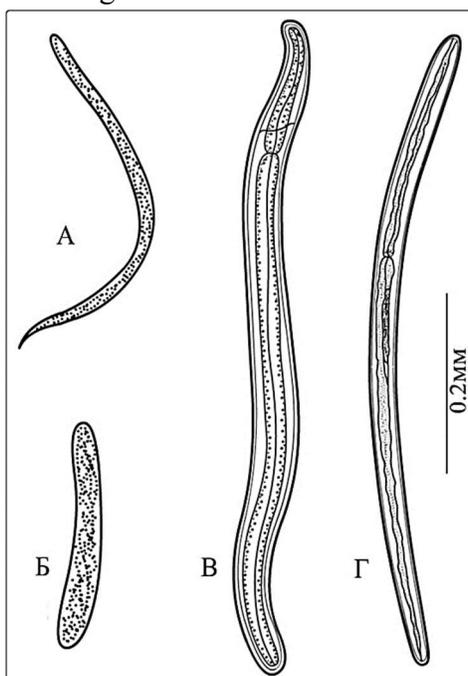
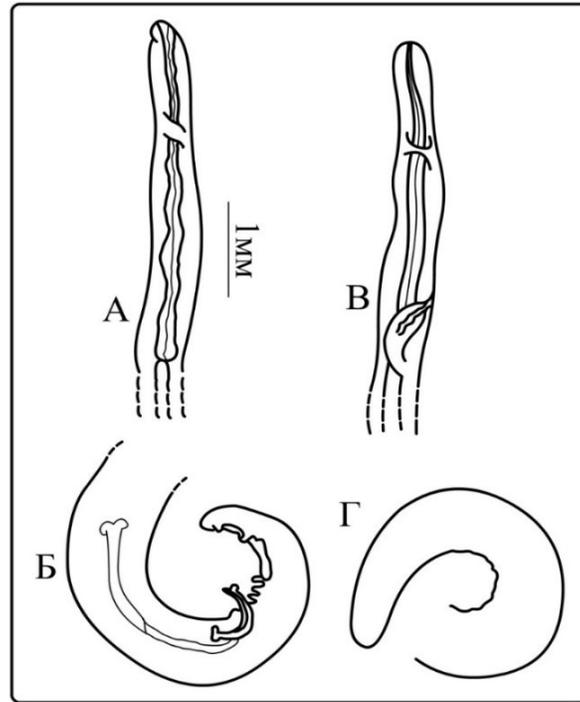


Fig.1. *Onchocerca lienalis* (Stiles, 1892): successive stages of development of larvae in the body of midges: A-microfilaria from the intestines of midges; B-larva in the form of a "sausage" from the pectoral muscles of the midge; B-larva in the process of 2 molts from the pectoral muscles of the midge; G-infective larva from the lower lip of the midge (original).

The detected nematodes are represented by males and females: males are 30-45 mm long and 0.038-0.064 mm wide; females 180-260 mm long and 0.160-0.230 wide. mm. Microfilariae isolated from the skin were 0.240–0.258 mm long and 0.002–0.005 mm wide (Fig. 2).



Rice. 2. *Onchocerca lienalis* (Stiles, 1892): A - anterior end of the male, laterally; B-tail end of the male; B - anterior end of the female, laterally; G-tail end of the female (original)

The infestation of midges by onchocercus larvae is dynamic in nature, associated with the phenology of the considered bloodsuckers. The maximum invasion in midges was noted in summer (19.1%). Infection of animal definitive hosts with onchocerci occurs when midges infested with larvae of these nematodes attack.

Features of the biology of *Stephanofilaria stilesi* Chitwood, 1934. Nematodes of the genus *Stephanofilaria* are known as parasites of the skin of artiodactyls. The genus includes four valid species: *Stephanofilaria dedoesi*, *S. assamensis*, *S. koeli*, *S. stilesi*. Two of them (*S. assamensis* and *S. stilesi*), parasitizing in the skin of cattle, were also noted in Uzbekistan [28,29,30].

The material of this study was our own collection of Diptera insects, potential intermediate hosts of the nematode *Stephanofilaria stilesi* in various regions of Uzbekistan. Insects were collected in the areas of concentration of cattle in the south and north-east of Uzbekistan during 2009-2015. The study of the collected insects showed that the larvae of *stephanofilaria* were found only in three species of blood-sucking flies of the family Muscidae (Table 3).

Table-3.

Infestation of flies with larvae of *stephanofilaria* in natural conditions of Uzbekistan

View	Regions	
	Southern	Northeastern

	Examined specimen.	Infected specimen. and %	Examined specimen.	Infected specimen. and %
<i>Lyperosia irritans</i>	2010	24(1.2%)	1975	20(1.0%)
<i>Lyperosia titillans</i>	2645	85(3.2%)	1805	38(2.1%)
<i>Stomoxys calcitrans</i>	960	9(0.93%)	991	9 (0.9%)
Total	5615	0.93-3.2%	4891	0.9-2.1%

Analysis of Table 3 shows that among the studied flies *Lyperosia irritans*, the infested larvae of *Stephanofilaria* were 1.0% and 1.2%; Consequently, bloodsucking flies are sufficiently infested with larvae of *stephanofilaria* both in the south and northeast of Uzbekistan.

Nematode larvae in flies are found in the warm season. The maximum infestation of blood-sucking flies was registered: the first - at the end of May and at the beginning of June, the second - in September. During this period, there is a slight fluctuation in the invasion of flies by larvae of *stephanofilaria*.

The dynamics of infestation of flies (*Lyperosia irritans* and *L. titillans*) by larvae of *stephanofilaria* varied by season within 0.1–3.2%, which probably depends on the ecology of the blood-sucking flies of this species. In the dynamics of the number of *lyperosia* in Uzbekistan, we noted two rises (spring and autumn). *Lyperosia* are infected with *Stefano-filariae microfilariae* in spring, summer and autumn. The number of *lyperosia* during the day is not the same. Most active and numerous in the morning (7-10) and evening (18-20) hours. In hot weather, flies, as a rule, leave the object of food and only single specimens are found on animals.

Consequently, in Uzbekistan, cattle are infected with *stephanofilaria* in spring, summer and early autumn, i.e. from the end of April to October.

A morphological study of the larvae found in *Lyperosia irritans*, *L. titillans*, and *Stomoxys calcitrans* showed that the body of infective larvae is elongated, the head and tail ends are rounded. At the head end is a crown of 18-19 spines. Well, the nerve ring and esophagus are expressed. The length of the infective larva reaches 0.60-0.90 mm, the width is 0.037-0.050 mm. The nerve ring is located at a distance of 0.022-0.050 mm from the head end. The length of the esophagus is 0.070-0.096 mm. Along with invasive larvae, we also found them at different stages of development.

Microfilariae isolated from the egg membrane (from the uterus of the parasite) have a length of 0.020-0.024. The body is filiform, the head end is blunt, the posterior end is pointed. From the results of studies and analysis of literature data, it logically follows that the life cycle of the nematode *Stephanofilaria stilesi* proceeds with the participation of bloodsucking flies (*microfilariae* enter the body of *Lyperosia* and *Stomoxys* flies with blood from skin wounds (infested animals) during bloodsucking (Fig. 3).

In the body of bloodsuckers, *microfilariae* go through a number of stages of development. In this case, a morphobiological restructuring of a number of organs and systems occurs. The larvae molt twice and become invasive. With repeated bloodsucking from the proboscis of

bloodsucking flies, invasive larvae of the nematode penetrate into the organism of the definitive host, where they develop to sexual maturity. To confirm this point of view, we carried out experimental infection of calves with invasive larvae of stephanofilaria collected from *Lyperosia irritans*, *L. titillans*, and *Stomoxys calcitrans*.

The experiments were carried out from June 15 to October 20, 2010 according to the following scheme: two calves (calves No. 1 and No. 2) were given orally 350 invasive larvae of stenofilaria; two animals (calves No. 3 and No. 4) were infected by introducing 350 infective larvae intradermally into the inner surface of the ears; two bulls (No. 5 and No. 6) were injected with 350 larvae intradermally into the region of the lower part of the peritoneum (umbilical cord). Goby No. 7 served as the general control.



Rice. Fig. 3. Scheme of the development cycle of *Stephanofilaria stilesi* (Chitwood, 1934) according to V.M. Ivashkin et al., 1971, with our changes.

Animals of the experimental and control groups were kept under similar conditions, excluding spontaneous infection with stephanofilaria. Systematically conducted observations on these animals.

Studies have shown that after 60 days (08/15/2010) in calves No. 3 and No. 4, bleeding ulcers were observed on the inner surface of the left ear. We noted a similar picture in experimental animals No. 5 and No. 6 75 days (08.30.2010) after infection. Ulcers were noted on the lower part

of the peritoneum, near the umbilical cord and the inner surface of the auricles. An accumulation of bloodsucking flies, mainly *Lyperosia irritans* and *L. titillans*, was observed on the surface of the ulcers. In the reticular layer of the skin of these animals, sexually mature nematodes (males and females) and a large number of microfilariae, as well as a small amount of parasite eggs, were found.

Affected areas of the skin of cattle, both in natural and experimental conditions, were noted in the ears and in the lower wall of the abdomen. The affected areas are devoid of hair, the wounds are covered with blood and serum. Mature nematodes isolated from wounds are quite small.

The mouth is surrounded by a crown of 18-19 spines. The cuticle is transversely striated. The body length of parasites ranges from 3.2–36 mm to 5.8–6.2 mm.

Blood-sucking flies *Lyperosia irritans*, *L. titillans* and *Stomoxys calcitrans* participate in the circulation of the invasion, the latter species is registered as a new vector of invasion in Uzbekistan.

Recognizing the dependence of the formation of the life cycles of filariata on the nature of their localization in the body of the final host, we note that, with rare exceptions, mammalian filariae have adapted to parasitism in closed organs and systems of animals.

The females of these groups are viviparous. They give birth to microfilariae. As exceptions, representatives of the *Stephanofiliariidae* family can be presented, which parasitize in the skin (peritoneum, withers, auricles) of animals. *Stephanofilaria* females are oviparous. At the same time, microfilariae are able to emerge from a thin egg shell in the places of laying, i.e. in parasitic wounds. Intermediate hosts are flies of the *Muscidae* family. Together with the blood of cattle, the flies swallow microfilariae and parasite eggs.

It should be emphasized that viviparous filariases have another unique feature - the born microfilariae of cavity parasites have the ability to actively penetrate into the lymphatic and blood vessels of the host, the mechanisms of which scientists have not yet been able to explain. Nevertheless, in the considered nematodes, the evolution of life cycles is clearly visible, which is expressed both in their simplification and in the replacement of the intermediate host. The replacement of the intermediate host of viviparous filariases contributed to their isolation from the direct impact of environmental factors in all phases of their development.

Biology of *Stephanofilaria assamensis* Pande, 1936. Nematodes *Stephanofilaria assamensis* Pande, 1936 are quite common among cattle in the south and northeast of Uzbekistan and cause a chronic disease of animals.

Under the influence of parasites in various parts of the skin of animals, bleeding wounds are formed (on the shell of the ears, the back of the head and peritoneum), which negatively affect the growth, development and decrease in the productivity of cattle.

The material for this work was the collection and study of flies of the family *Muscidae* of the fauna of Uzbekistan from the plain and foothill zones of the Surkhandarya, Kashkadarya, Tashkent, Syrdarya, Jizzakh regions. In places where cattle are concentrated, a large number of blood-sucking flies of the genera *Stomoxys*, *Haematobia* and *Lyperosia* have been collected and

studied. When studying the collected blood-sucking flies, we found that they are represented by 5 species belonging to 3 genera (Table 4).

Table-4.

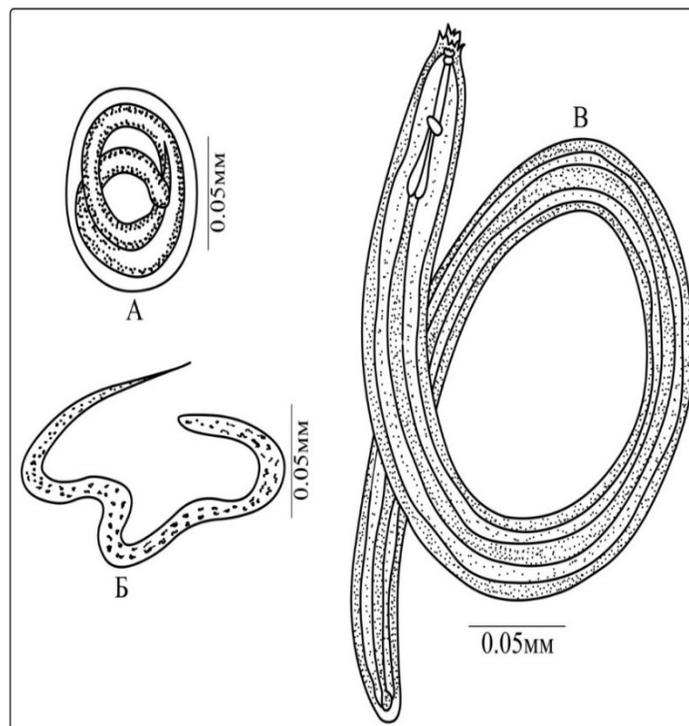
Infection of blood-sucking flies of the Muscidae family with larvae of *Stephanofilaria assamensis* in the natural conditions of Uzbekistan

Types of blood-sucking flies	Researched		
	Total, ind.	Infected, number and %	Intensity of invasion
<i>Stomoxys calcitrans</i>	1005	–	–
<i>Haematobia stimulans</i>	1002	–	–
<i>Haematobia atripalpis</i>	1010	21(2.1%)	5 – 103
<i>Lyperosia irritans</i>	1009	12 (1.2%)	3 – 15
<i>Lyperosia titilans</i>	1007	16 (1.5)	3 – 9

Of the 5 species of flies studied, the larvae of *Stephanofilaria assamensis* were infested: *Haematobia atripalpis*-2.1%, *Lyperosia irritans*-1.2% and *Lyperosia titillans*-1.5%. The intensity of the invasion of individual individuals, infected flies was 3-103 ind.

Infective larvae of *stephanofilaria* in these bloodsuckers are found in the warm season, from May to October. The maximum infestation of bloodsucking flies was registered at the end of May and at the beginning of September. From this it should be assumed that cattle are infected with *stephanofilaria* in spring, summer and early autumn. According to the degree of infestation, the flies *Haematobia atripalpis* occupy the leading position. Probably, this species plays the role of an obligate intermediate host of *Stephanofilaria assamensis* in the conditions of Uzbekistan. This can be evidenced by a significant infestation of *Haematobia atripalpis* (2.1%) by the larvae of this nematode, compared with the infestation of the lesser and southern sturgeons.

A morphological study of the larvae found in *Haematobia atripalpis*, *Lyperosia irritans* and *Lyperosia titillans* showed that the invasive larvae of *stephanofilaria* are characterized by the following features: the larva is 0.810 - 1.120 mm long, 0.040 - 0.050 mm wide. its body tapers towards the anterior and posterior ends. At the head end there is a small ledge in the form of a crown, seated with 23 - 24 spines. A fairly well-defined nerve ring is located at a distance of 0.026 - 0.048 mm from the anterior end of the body (Fig. 4).



Rice. 4. *Stephanofilaria assamensis* Pande, 1936: A-egg with larva; B-microfilaria; B-infective larva (original).

It is logical to note that microfilariae enter the body of flies together with blood from a skin wound of infected cattle during bloodsucking. In the body of bloodsuckers, microfilariae go through a number of stages of development, where morpho-biological restructuring of organs and systems occurs. The larvae molt twice and become invasive. With repeated bloodsucking from the proboscis of bloodsucking flies, the invasive larvae of *Stephanofilaria* penetrate into the organism of the definitive host, where they develop to sexual maturity. This is also evidenced by the results of our research.

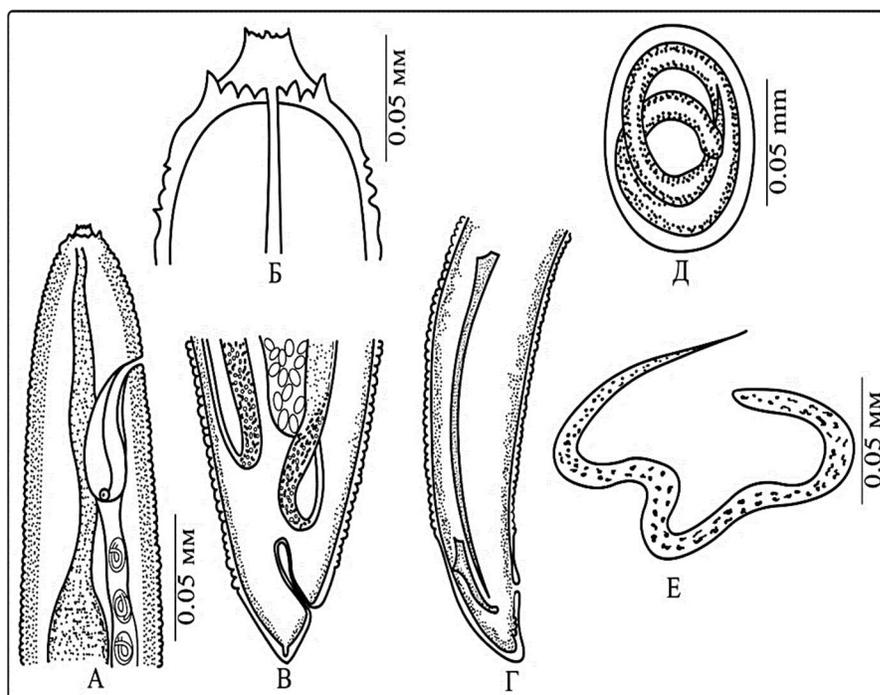
To clarify this point of view, we attempted to reproduce the life cycle of *Stephanofilaria assamensis* with inoculation of infective larvae collected from *Haematobia atripalpis*, *Lyperosia irritans* and *L. titillans*.

Experimental infection of animals was carried out according to the following scheme: two calves (calves No. 8 and No. 9) were infected by introducing 275 copies of invasive larvae intradermally into the neck area. Goby No. 10 served as control.

The animals of the experimental groups were infected on June 25, 2010. Animals of the experimental and control groups were kept under similar conditions, excluding spontaneous infection with *Stephanofilaria*. Systematically conducted observations on these animals.

Studies have shown that after 55-65 days in calves No. 8 and No. 9, the formation of bleeding ulcers on the outer surface of the auricles was observed. An accumulation of bloodsucking flies, mainly *Haematobia atripalpis*, *Lyperosia irritans* and *L. titillans*, was observed on the surface of the ulcers. In the reticular layer of the skin of these animals, sexually mature

nematodes (males and females) and a large number of microfilariae, as well as insignificant eggs of the parasite, were found. Mature nematodes isolated from wounds are rather thin, females are larger and longer than males (Fig. 5).



Rice. 5. *Stephanofilaria assamensis* Pande, 1936: A. Anterior end of female, laterally; B-head end; V-caudal end of the female, laterally; G-tail end of the male; D-egg with larva; E-microfilaria (original).

The cuticle is transversely striated. The anterior and posterior ends are bluntly rounded, with a terminal cuticular ridge surrounding the mouth. The crest bears a number of small spines, the number of which is 23-24. The second circle consists of larger spines. The esophagus begins behind the mouth.

Male. Body length 3.2-4.5 mm, maximum width 0.10-0.12 mm. The nerve ring surrounds the esophagus from its middle at a distance of 0.06-0.08 mm from the anterior end. The length of the esophagus is 0.012-0.16 mm. The tail end is rounded.

Caudal papillae are numerous. Spicules are unequal. The length of the left spicule is 0.16–0.19 mm; the length of the right spicule is 0.04–0.05 mm. Rudimentary rudimentary.

Female. Body length 8.2-11.0 mm, maximum width 0.16-0.20 mm. The distance from the head end to the nerve ring is 0.07-0.09 mm. The length of the esophagus is 0.19-0.22 mm.

The vulva is located behind the nerve ring, at a distance of 0.10-0.12 mm from the head end. The oviduct divides into two uteruses running parallel to the posterior end of the body. The found eggs are fully developed, thin-walled and have dimensions of 0.036-0.040x0.023-0.030 mm. Length of larvae isolated from eggs 0.122-0.124 mm.

Analysis of the research results and literature data shows that the life cycle of *Stephanofilaria assamensis* in its distribution areas occurs with the obligatory participation of blood-sucking flies *Musca conducens*, *Musca planiceps*, *Lyperosia irritans*, *L. titillans* and *Haematobia atripalpis* [31,32,33,34]. The circle of intermediate hosts of the nematode *S. assamensis* was supplemented by another species *Haematobia atripalpis*. The last three species are noted by us as carriers of invasion for the first time.

Biology of *Thelazia rhodesi* (Desmarest, 1827). Telaziosis is a widespread invasion, starting mainly in the summer months. Calves of the current year of birth are most often affected. Animals have lacrimation, photophobia, keratitis, conjunctivitis, and sometimes loss of vision.

In order to study the fauna and biological characteristics of the telazia in various conditions of Uzbekistan, a helminthological examination of the eyes of about 10 thousand cattle was carried out according to the method of K. I. Skryabin. More than 20 thousand flies belonging to 16 species (*Lyperosia irritans*, *L. titillans*, *Stomoxys calcitrans*, *Musca autumnalis*, *M. dipecina*, *M. domestica*, *M. vitripennis*, *M. osiris*, *M. tempestiva*, *M. larvipara*, *M. lucidula*, *Musca sp.*, *Muscina stabulana*, *Pyrellia codaverina*, *Fania canicularis*, *Haematobia atripalpis*), in order to study the intermediate hosts of telazia in various regions of the republic.

According to the results of our research and analysis of literature data, 4 types of telazia have been identified in cattle of Uzbekistan: *Thelazia rhodesi*, *Th. gulosa*, *Th. skrjabini*, *Th. petrovi* [35,36,37].

These are small nematodes up to 21 mm in size. *Thelazia rhodesi* is considered the most pathogenic. Males have two unequal spicules. In females, the vulva opens in front of the body, they are viviparous. Nematodes of the other two species have smooth cuticles, while males have almost equal spicules. *Thelazia rhodesi* is localized in the conjunctival sac and under the third eyelid, while *Thelazia gulosa* and *Thelazia skrjabini* are located in the ducts of the lacrimal gland and the nasolacrimal canal.

Among telazia, the species *Thelazia rhodesi* is widespread in cattle in all regions of the republic. This parasite is localized in the conjunctival sac and under the third eyelid of cattle. Other types of telazia parasitize in the ducts of the lacrimal gland and the lacrimal-nasal canal.

In different regions of our republic, the total infection of a large cattle with telaziosis ranges from 5.8% to 30.7%. According to the results of our research, the causative agents of telaziosis are found in cattle at any time of the year. However, the peak of the disease occurs in summer and autumn. In this case, the summer increase in the disease is due to the infection of animals with the larvae of the telazia in the spring, and the autumn outbreak is due to the infection of animals with the larvae of the telazia in the summer and early autumn. From the above data, it can be seen that the incidence of telaziosis in animals varies depending on the seasons and regions of the country. For example, in the Syrdarya and Tashkent regions, cattle become infected with telaziosis by 0.3-13.7% in spring, by 1.6-14.9% in summer and by 26.2-72.5% in autumn. In the Jizzakh region and the Ferghana Valley, the total infection of cattle with telaziosis was 7.5-14.0% in spring, 8.2-16.6% in summer, 17.7-20.8% in autumn and significantly decreased in winter to 6.7-14.3%. In Navoi, Bukhara, Kashkadarya and

Surkhandarya regions, as well as in the Republic of Karakalpakstan, the overall incidence of cattle thelaziosis is 3.1-6.4% in spring, 6.9-13.8% in summer, 7.1-35.8% in autumn and 6.1-22.2% in winter.

Flies living in pastures are intermediate hosts of telasia and play an important role in the spread of causative agents of telasiasis, i.e. *Thelazias* are biohelminths that are spread by flies.

The intermediate hosts of *Thelazia rhodesi* are: *Musca larvipara*, *M. autumnalis*, *M. convexifrons* and *Morellia simplex*.

In various regions of Uzbekistan, it has been determined that as an intermediate host of *Thelazia rhodesi* species parasitizing in

livestock, fly species *Musca larvipara*, *M. domestica* and *Musca* sp. In some regions of the country, the infestation of these flies by *Thelazia rhodesi* larvae ranges from 0.2% to 0.7%.

It is known that female telazia lay live larvae on the mucous membrane of the eye in animals. Intermediate hosts of these fly parasites become infected with larvae through the tears of cattle. The larvae develop in the body of flies within 15-30 days and pass into the invasive stage. Invasive larvae enter the stomach of the flies and then move on to the proboscis. When such flies land near the eyes of cattle, the infective larvae emerge from the proboscis of the fly and enter the eye of the cattle, infecting it with thelaziosis. The length of the larvae that got into the eye of the animal is 6 mm, and after 20-45 days they turn into adult males and females.

Infection of cattle with larvae of thelaziosis pathogens occurs mainly through intermediate hosts in water sources on summer pastures. The life expectancy of calves in cattle is from several months to a year.

Thelaziosis is a seasonal disease that occurs in the summer and in winter. In the eyes and in the nasal cavity of cattle, telazias can be found throughout the year. But more often they can be found in July and September.

It is known that female telazia lay live larvae on the mucous membrane of the eye in animals. Intermediate hosts of these parasites - flies - become infected with larvae through the tears of cattle. The larvae develop in the body of flies within 15-30 days and pass into the invasive stage. Invasive larvae enter the stomach of the flies and then move on to the proboscis. When such flies land near the eyes of cattle, the infective larvae emerge from the proboscis of the fly and enter the eye of the cattle, infecting it with thelaziosis. The length of the larva that got into the eye of the animal is 6 mm, and after 20-45 days they turn into adult males and females.

Features of the biology of *Parabronema skrjabini* (Rassowska, 1924). *Parabronema skrjabini* parasitizes the abomasum and thin intestine of cattle and other ruminants.

Despite the wide distribution of the parasite among cattle and other ruminants in certain regions of the republic, the features of the biology and epizootology of the causative agent of parabronemosis have not been studied enough. In this regard, for many years, while studying the ecological and faunal characteristics of helminths of ungulates, including cattle in various conditions of Uzbekistan, we also studied some issues of the biology and epizootology of the causative agent of parabronemosis.

In order to identify the intermediate host of *Parabronema skrjabini* in various biocenoses of Uzbekistan, in different seasons of the year, we collected and studied 15552 specimens. flies belonging to 17 species (Table 5).

The species composition of Diptera was determined according to A.A. Stackelberg [38]. As a result of the research, we found that in the conditions of Uzbekistan, the role of intermediate hosts of *parabronema* is performed by 3 species of blood-sucking flies - the southern cow fly (*Lyperosia titillans*), the small cow fly (*Lyperosia irritans*) and the autumn fly (*Stomoxys calcitrans*). Autumn Stinger is registered by us as a new intermediate host.

Table -5.

The study of various species of flies for infection with their larvae of *Parabronema skrjabini*, a parasite of cattle in the conditions of Uzbekistan

№	Fly species	Number of flies, ind..		EI, %	II, copy.
		Explored	Infected		
1	2	3	4	5	6
1.	<i>Lyperosia titillans</i> Bezzi	3551	118	3,3	1-20
2.	<i>Lyperosia irritans</i> L.	2433	89	3,7	1-21
3.	<i>Stomoxys calcitrans</i> L.	838	6	0,7	1-3
4.	<i>Musca autumnalis</i> De-Geer.	451	–	–	–
5	<i>M. direcina</i>	112	–	–	–
6.	<i>Musca lucidula</i> Lw.	2326	–	–	–
7.	<i>Musca domestica vicina</i> Meq.	653	–	–	–
8.	<i>Musca albina</i> Wd.	667	–	–	–
9.	<i>Musca vitripennis</i> Mg.	87	–	–	–
10.	<i>Musca osiris</i> Wd.	144	–	–	–
11.	<i>Musca tempestiva</i> Flln.	1827	–	–	–
12.	<i>Musca larvipara</i> Portisch.	745	–	–	–
13.	<i>Musca</i> sp.	658	–	–	–
14.	<i>Muscina stabulans</i> Flln.	565	–	–	–
15.	<i>Wohlfahrtia indigena</i> Vill.	452	–	–	–
16	<i>Pyrellia cadavarina</i>	27	–	–	–
17	<i>Fania canicularis</i>	16	–	–	–
	Total	15552			

The total infestation of blood-sucking flies with *parabronema* larvae was 3.3% in *Lyperosia titillans*, with an invasion intensity of 1-20 specimens, in *Lyperosia irritans*-3.7%, with an invasion intensity of 1-21 specimens, in *Stomoxys calcitrans*-0.7 %, with an invasion intensity of 1-3 specimens.

Phenology, seasonal dynamics, diurnal rhythm of activity, the proportion of individual fly species throughout the year are not the same and vary depending on the landscape, geographical

and climatic conditions of Uzbekistan. Thus, in the desert zone, the years of the most widespread flies begin mainly at the end of March and end in November and December [39]. The first rise in the number of flies in the desert zone is observed in April and May, the second - in September and October. The increase in the population of flies is due to the optimal conditions established during these periods, the decrease in the number of insects in summer is associated with an increase in the absolute maxima of air temperature to +35-40°C and more, and soil temperature to +60-70°C.

The dynamics of infestation of flies by *Parabronema* larvae also varied depending on the season. The maximum infestation of these flies was noted at the end of April-May (*Lyperosia titillans*-5.5-8.6%, *Lyperosia irritans*-2.7-7.5%, *Stomoxys calcitrans*-1.1%. In June-July, an unknown significant decrease in invasion (*Lyperosia titillans*-1.3-1.4%, *Lyperosia irritans*-0.7-1.4%)., *Lyperosia irritans* -6.5-12.5%, *Stomoxys calcitrans* -0.5%).

We first discovered *parabronema* infective larvae in intermediate hosts in the second decade of April, which is 2 months earlier than in other natural conditions of the CIS.

Therefore, the role of the obligate intermediate host of the nematode *Parabronema skrjabini*, the causative agent of *parabronemosis*, under the conditions of Uzbekistan, is performed by blood-sucking flies, the southern cow's stallion (*Lyperosia titillans*) and the lesser cow's stallion (*Lyperosia irritans*). Infection of flies with *parabronema* eggs occurs in spring, early summer and autumn, but mainly at the end of April - in May and in September-October.

Flies become infected with *parabronema* eggs only in the early stages of their development, i.e. parasite females lay eggs, which are excreted into the external environment with the faeces of animals. Female flies also lay their eggs only on animal feces. After about a day, larvae hatch from the eggs of the flies, which feed on the feces of animals and at the same time swallow the eggs with *parabroneme*. Then, in the intestines of the fly larvae, larvae hatch from the eggs of the *parabronemes*, which actively penetrate into the body cavity of the insects. Further, simultaneously with the metamorphosis of flies, the development of larvae of *parabronemes* occurs, which become invasive only when the flies reach the imaginal form [40].

Infection of cattle with *parabronemes* occurs through the mouth. At the same time, infected flies, crawling up to the wet and warm lips of the animal, throw out *parabronemal* infective larvae through the proboscis (Fig. 6). Cattle can also become infected with these parasites by ingesting flies infested with nematode larvae along with food. In the digestive tract, *parabronema* infective larvae penetrate the abomasum mucosa, grow, molt, and eventually develop into sexually mature *parabronema*.



Fig.6. Scheme of the development cycle of *Parabronema skrjabini* (Rassowska, 1924) according to Ivashkin et al., 1983, with our additions

To establish the distribution and seasonal dynamics of the causative agent of parabronemiasis, we studied the gastrointestinal tracts of 496 cattle using the method of complete helminthological dissections. The research was carried out by us directly in the farms of Bukhara, Jizzakh, Kashkadarya, Navoi, Surkhandarya, Khorezm regions and the Republic of Karakalpakstan, as well as at meat processing plants. The results of the research showed that the pathogens of parabronemiasis in cattle are not equally distributed in certain regions of the republic. The total infection of cattle with parabronemiasis was 4.0-56%. The causative agent of the disease is most widely distributed among cattle in the biogeocenoses of the northwestern and central parts of the republic. Cattle are more infected with parabronemes in the farms of the Bukhara and Navoi regions, as well as the Republic of Karakalpakstan.

Thus, under the conditions of Uzbekistan, pathogens of parabronemiasis are found in cattle at any time of the year, but the invasion rate increases mainly in spring, autumn and winter. The data obtained by us will serve as a scientific basis for planning and carrying out therapeutic and prophylactic measures against parabronemiasis of cattle and other ruminants in specific biocenoses of Uzbekistan.

Features of the biology of *Setaria labiatopapillosa* (Alessandrini, 1838). Setariosis is a helminthic disease of cattle caused by several species of nematodes, but more often by the species *Setaria labiatopapillosa* from the family Setariidae of the suborder Filariata.

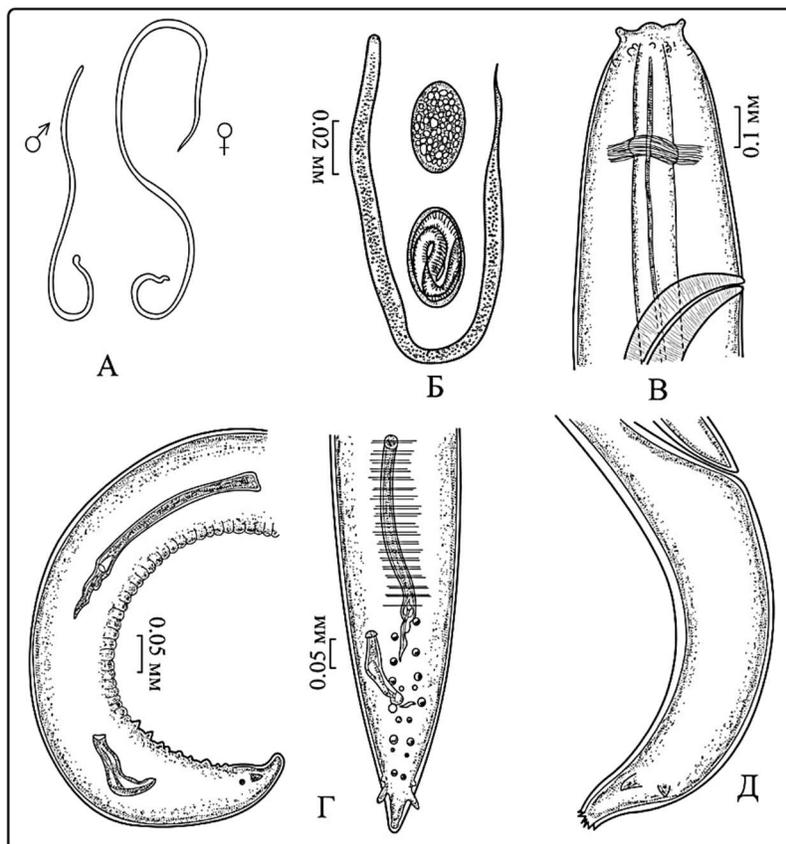
Sexually mature setaria parasitize in the abdominal cavity, attaching to the serous membrane of the intestine, liver, and diaphragm.

Setaria are thin grayish-white nematodes, males up to 5 cm long, females up to 10 cm. There are two semicircular elevations at the head end. Males have two unequal spicules. In females, the genital opening is located near the head end (Fig. 7).

Setaria-Setaria labiatopapillosa are widespread in cattle in the biogeocenoses of Uzbekistan, the diseases they cause are characterized by a chronic course. The increased interest in this nematode species observed in recent years has led to a more in-depth study of the biology of *Setaria labiatopapillosa* in Uzbekistan [41,42,43,44].

The material for this work was the results of faunistic and experimental studies on the biology of *Setaria labiatopapillosa*, a parasite of cattle in terrestrial biocenoses of Uzbekistan. At the same time, 496 heads of cattle were investigated by the method of complete helminthological autopsies, directly in the farms of Bukhara, Jizzakh, Kashkadarya, Navoi, Surkhandarya, Khorezm regions and the Republic of Karakalpakstan, as well as at meat processing plants.

The results of our research and data obtained by other authors show that setarii are registered in all surveyed regions of the republic. The degree of infestation of animals with setariosis in some regions of the republic varied in the aisles from 16.6 to 75.7%, with an intensity of invasion of 1-31 ind. The highest extensiveness of setariosis invasion in cattle was noted in the northwestern and central parts of the republic, lower in the eastern and northeastern parts of Uzbekistan. Sexually mature setaria in places of localization lay eggs, from which microfilariae larvae then hatch. The larvae then migrate into the blood vessels and become available to insects. In the body of insects, the larvae become infective in 15-20 days. Such insects, during the period of bloodsucking on animals, inoculate infective larvae. Then the larvae migrate through the tissues of the host into the abdominal cavity and after 6-8 months. become sexually mature.



Rice. 7. *Setaria labiatopapillosa* (Alessandrini, 1838): A-male and female life-size; B-microfilaria and eggs from the uterine cavity; B-anterior end of female body with vulva; G-tail ends of the male, ventrally and laterally; D-tail end of female with anus. (according to Scriabin, Petrov, 1964, modified).

Setaria are known to develop with the participation of intermediate hosts, blood-sucking Diptera of the Culicidae and Muscidae families. Spontaneous infestation of Diptera by Setaria larvae was studied in 4787 specimens. collected in farms from various regions of Uzbekistan, which are unfavorable for setariosis, in spring, summer and autumn. Insects were collected with test tubes and an exhaustor from cattle. The study of these insects showed that microfilariae and larvae of the corresponding developmental stages were found in *Stomoxys calcitrans* (Muscidae), mosquitoes (Culicidae)-*Culex pipiens*, *Aedes caspius*, *Anopheles maculipennis* (Table 6). In insects that sucked on the blood of cattle, microfilariae were noted in the intestines, and larvae in various stages of development - in the pectoral muscles. Infective larvae were localized in the proboscis of insects. Larvae of *Setaria labiatopapillosa* were found in flies *Stomoxys calcitrans* (0.9%) and in mosquitoes *Aedes caspius* (0.3%).

Table-6.

**Natural infestation of blood-sucking Diptera larvae
setaria in the conditions of Uzbekistan**

View	Researched, copy.	Infected, %
		<i>Setaria labiatopapillosa</i>
Muscidae: <i>Stomoxys calcitrans</i>	989	0.9
Culicidae: <i>Culex pipiens</i>	867	-
<i>Aedes caspius</i>	2336	0.3
<i>Anopheles maculipennis</i>	595	-

Invasive larvae of these *Setaria* species are recorded in bloodsuckers during the warm season (May-September). It can be assumed that cattle are infected with setaria in late spring and summer. This process continues until the onset of cold weather..

Conclusion. Thus, the article presents original data on the biological characteristics of some species of nematode parasites of cattle that develop with the participation of Diptera in the biocenoses of Uzbekistan.

In cattle of Uzbekistan, 71 species of helminths are registered, of which 12 species belong to the class Cestoda Rudolphi, 12 species to the class Trematoda Rudolphi, 1808 and 47 species to the class Nematoda Rudolphi, 1808. their adaptation to a variety of environmental conditions, contributing to the development of parasites in all phases of life cycles. Of the identified 47 species of cattle nematodes in Uzbekistan, 10 species develop with the participation of dipterous insects.

Biological features are highlighted - *Onchocerca lienalis* (Stiles, 1892), *Stephanofilaria stilesi* Chitwood, 1934, *Stephanofilaria assamensis* Pande, 1936, *Thelazia rhodesi* (Desmaresi, 1827), *Parabronema skrjabini* (Rassowska, 19240) and *Setaria labiatopapillosa* (Alessandrini, 1838) - parasitic bovine livestock developing with the participation of dipterous insects in various biocenoses of Uzbekistan.

Consequently, the participation of the corresponding hosts in the life cycles of nematodes acquires a special biological meaning in the implementation of coenotic relationships between the host and the parasite. It is quite obvious that the development of any preventive measures can be based only on a detailed knowledge of the biological characteristics of both the parasite and the host, which ensure their contact in the external environment.

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