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INTRASPECIFIC MORPHOLOGICAL VARIATION IN FREE-LIVING STAGES OF *STRONGYLOIDES PAPILLOSUS* (NEMATODA, STRONGYLOIDIDAE) PARASITIZING VARIOUS MAMMAL SPECIES

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Intraspecific Morphological Variation in Free-Living Stages of *Strongyloides papillosus* (Nematoda, Strongyloididae) Parasitizing Various Mammal Species. Boyko, O. O., Gugosyan, Y. A., Shendryk, L. I., Brygadyrenko, V. V. — *Strongyloides papillosus* Wedl, 1856 is one of the most widespread nematodes parasitic in domestic animals. This species has been recorded on almost all continents of the planet. It parasitizes the small intestine of rabbits, sheep and cattle. At laboratory conditions, this species can also infect guinea pigs. Morphological variability of *S. papillosus* in relation to host species has not yet been studied. Our research showed that L_1 and L_2 of *S. papillosus* reached their maximum size in all parameters in guinea pigs: for L_1 — length and width of the body, length of esophagus and intestine; for L_2 — width of body and length of intestine. L_3 of *S. papillosus* had statistically reliable differences in almost all parameters (except the length of intestine) when parasitizing goats and rabbits. For L_3 the width of the body and the length of the tail end, we determined differences between *S. papillosus* infecting rabbits and guinea pigs, and also goats and guinea pigs. Male *S. papillosus* were observed to have statistically reliable differences in all morphological parameters for goats and rabbits, and also for rabbits and guinea pigs. Practically all measurements (except the length of the tail) were larger for *S. papillosus* in guinea pigs and goats than those in rabbits. Free-living females statistically differed by larger size in goats and rabbits. The smallest dimensions were determined for all free-living stages of *S. papillosus* in goats (except males). The largest size in most cases was determined for larval stages of *S. papillosus* in guinea pigs.
Key words: guinea pigs; morphometric parameters, rabbits, Ruminantia, *Strongyloides papillosus*.

Introduction

One of the most widespread nematode parasites of farm animals is *Strongyloides papillosus* Wedl, 1856. This species has a very wide geographical distribution and is often found during parasitological investigations. In 2014–2016, in Slovakia, the examination of 30 farming enterprises with dairy goats (944 individuals) found *S. papillosus* parasitic nematodes in the small intestine. Eggs of *S. papillosus* were found in the feces of 14.1 % of goats (Babják et al., 2017). The occurrence of *S. papillosus* was also proved by the studies in Kenya (Kanyari et al., 2010; Peter et al., 2015). During the examination of 110 calves in Kenya, eggs of the helminth were found in 5.4 % of cases. A study in Bangladesh found *S. papillosus* in 8.5 % of 117 samples of feces of cattle from a state dairy farm (Paul et al., 2016). The analysis of 143 samples of feces of cattle in Algeria in 2013–2014 found *S. papillosus* in 1.3 % of cases (Moussouni et al., 2018). Over the analysis of 185 samples of feces in the suburbs of Bangkok in 2014, *S. papillosus* was found in 16.8 % of goats (Azrul et al., 2017). During the examination of some enterprises in the territory of steppe Prydniprovia (Ukraine) in 2015, the infection of *Bos taurus* with *S. papillosus* was determined to be up to 91.7 %, and the level of the infection reached 850 eggs per 1 g of feces (Boyko, 2015). According to our data, the prevalence of the parasitic infection among cattle in different regions of Ukraine ranges from 20–80 %, the most affected by the parasite being young cattle under one year. The peculiarities of the development and morphology of helminths in different types of hosts were studied by Audebert et al. (2004), Eberhardt et al. (2008), Gugosyan et al. (2018, 2019) and others. Eberhardt et al. (2008) studied the populational differences of *S. papillosus* in cattle and sheep. Gugosyan et al. (2018) described in detail differential morphometric peculiarities of *S. westeri* in horses, determined the increase in the length and width of the bodies of the larvae in relation to changes of phases, and also the forming of filarial esophagus in the third stage larvae and decrease in the length of intestine. The authors describe the morphometric features of four species of *Strongyloides* parasitising in domestic animals of Ukraine. It was proposed to differentiate the types of *Strongyloides* by the ratio of various morphometric indices (Gugosyan et al., 2019).

Strongyloides are geohelminths, it's able to parasitize in the host organism and lead a free way of life, developing and multiplying outside the body of the animal. These nematodes have an unusual for most helminths life cycle, which involves two types of development — homogonic (direct) and heterogonic (indirect) (Olsen, 1974). By the direct developmental path, a rhabditiform larva emerges from the egg, it molts and after 2–3 days turns into filarial (invasive) larvae, under indirect path, it gives rise to the generation of free-living males and females. Under optimal environmental conditions, homogonic and heterogonic cycles occur simultaneously (Basir, 1950; Nwaorgu, 1983; Lyons & Tolliver 2015).

The possibility of infection of rabbits *S. papillosus* is described by scientists, however, the metric parameters of the larvae have not been established (Neilson & Nghiem, 1974; Taira et al., 1974; Nwaorgu & Connan, 1980; Nakamura & Motokawa, 2000; Kobayashi & Horii, 2008).

The objective of the present study was to determine the impact of host (goat, rabbit or guinea pig) on morphological variability of the free-living stages of *S. papillosus*.

Material and methods

The studies were conducted in 2017–2018 at the Department of Parasitology and Veterinary-Sanitary Examination of the Dnipro National Agrarian-Economic University. The material was sampled from feces obtained from animals spontaneously infected with *S. papillosus*. Over the experiment, we selected samples of feces of goats, *Capra hircus* (Linnaeus, 1758), and rabbits, *Oryctolagus cuniculus* (Linnaeus, 1758), from enterprises in Dnipropetrovsk Region. To avoid contamination of the material with free-living nematodes, the samples of feces of goats and rabbits were taken directly from the rectum. The cultivation and obtaining of free-living stages of development was conducted in a thermostat at the temperature of 23–25 °C over 1–7 days. The separation of the larvae and free-living generation adults of *S. papillosus* was made using the Baermann technique, and the identification was conducted using morphological parameters (Van Wyk et al., 2004; Van Wyk & Mayhew, 2013).

To study the potential of *S. papillosus* to parasitize guinea pigs, *Cavia porcellus* (Linnaeus, 1758), and determine the morphometric parameters of free-living stages, we conducted an experiment in which guinea pigs (n = 15) were selected according to the principle of pair analogues, considering age, body weight (350–400 g), and general development. Formed two groups of animals control (n = 5) and experimental in which the infection was carried out (n = 10). The guinea pigs were reared at laboratory conditions and over 45 days have been examined daily for diseases (including helminthiasis). The animals in the experiments were kept in the vivarium. They had free access to water and food. Throughout the experiment, their complete and uniform diet was composed of grain and succulent feed. The physiological condition of the laboratory guinea pigs was monitored daily through clinical observation and inspection.

The cultures of larvae of *S. papillosus* from the rabbits were put in a test tube and centrifuged during one minute at 1500 rotations a minute. Thereafter, the sediment with larvae was put in sterile test tube, and then a physiological saline was added. The procedure of cleaning was repeated many times until a pure larvae culture was obtained. The guinea pigs were infected perorally and subcutaneously with a culture of *S. papillosus* infective larvae. Over the experiment, the samples of feces were daily analyzed using the McMaster and Baermann

techniques. On the 28th day, we recorded eggs of *Strongyloides*. The sample material was selected and cultivated in a thermostat at 23–25 °C during 1–7 days. In the control group of animals, the appearance of eggs and larvae of *Strongyloides* were not observed. The metrical parameters of free-living stages of *S. papillosus*, obtained from the different species of animals, were determined using an eyepiece scale at the microscope under the magnification of ×100 and ×400. Photomicrographs were made using a Sigeta CAM MD-300 3 Mpix (China) digital camera. The study of metric parameters was carried out on live larvae and free-living *Strongyloides* generations. For all of the studied individuals, we determined total body length (Lb), maximum body width (Sb), length of esophagus (Lo), length of intestine (Le), length of tail (Lc), distance from the head end to vulva (Lv) and the number of formed eggs in the uteri (E) in free-living females, and length of spicules (Ls) in males. In total, 30 individuals of each stage of the development of *S. papillosus* were analyzed for each of the three host animal species. The data were compared using Tukey's test. Differences between the selections were considered statistically significant at $p < 0.05$. The data was analyzed in Statistica 8 (StatSoft Inc., USA). In the diagrams the small squares show the median, the large rectangles show the 25 % and 75 % quartiles, the vertical lines show 95 % of the variation, the stars and circles show the outliers. The medians are given in the text.

Results and discussion

The rhabditiform larvae had an elongated body, one (L_1) or two (L_2) bulb-like formations in the esophagus (fig. 1, *a*, *b*, *c*), the intestine was filled with a pigmented grainy mass, placed in two rows, the tail of L_1 varied in length and shape (fig. 1, *a*, *b*).

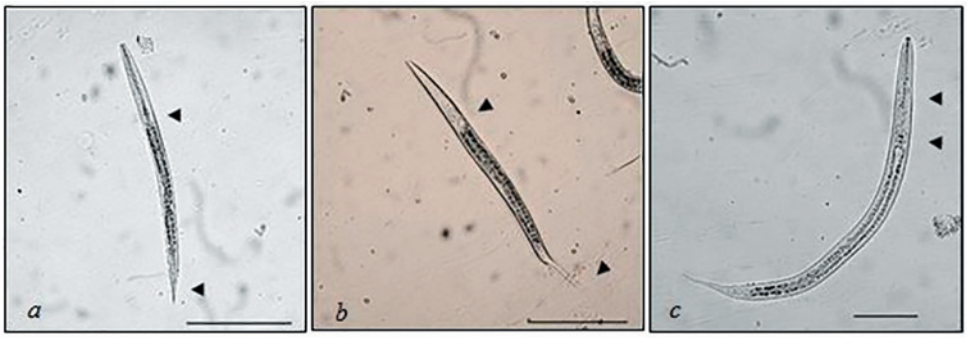

Fig. 1. Rhabditiform larvae of *S. papillosus*: *a* — L_1 from *C. hircus* (arrowheads indicate the bulb-like dilatations on the esophagus and the tail); *b* — L_1 from *O. hircus* (arrowheads indicate bulb-like dilatations on the esophagus and the tail); *c* — L_2 from *C. hircus* (arrowheads indicate two bulb-like dilatations); scale bars 100 μm.

During the analysis of the length of the larvae of the first stage (280–350 μm on average) obtained from goats, rabbits and guinea pigs, we determined a statistically reliable difference in the parameters of L_1 from goats and guinea pigs. The difference equaled about 70 μm. We also observed a statistically reliable difference in total length of this stage of larvae from rabbits and guinea pigs, 30 μm on average (fig. 2, *a*). The body width of L_1 from different types of hosts varied within 17.5–21.0 μm. Statistically significant difference was also observed in this parameter. A significant difference in body width was observed between L_1 from goats and those from guinea pigs (2.0 μm on average), and also between those from rabbits and those from guinea pigs (3.5 μm on average) (fig. 2, *b*).

The length of L_1 esophagus differed significantly for all the studied host species. About 2 μm was the difference in this character between the larvae from goats and those from rabbits. At the same time, the shortest length was determined among the larvae from the feces of rabbits (83 μm). The longest esophagus of L_1 was determined for the larvae from feces of guinea pigs (95 μm). Therefore, they significantly differed from the larvae from feces of goats and rabbits (fig. 2, *c*). The length of intestine of the first-stage larvae from rabbits and of those from guinea pigs showed a statistically significant difference (145–170 μm) (fig. 2, *d*).

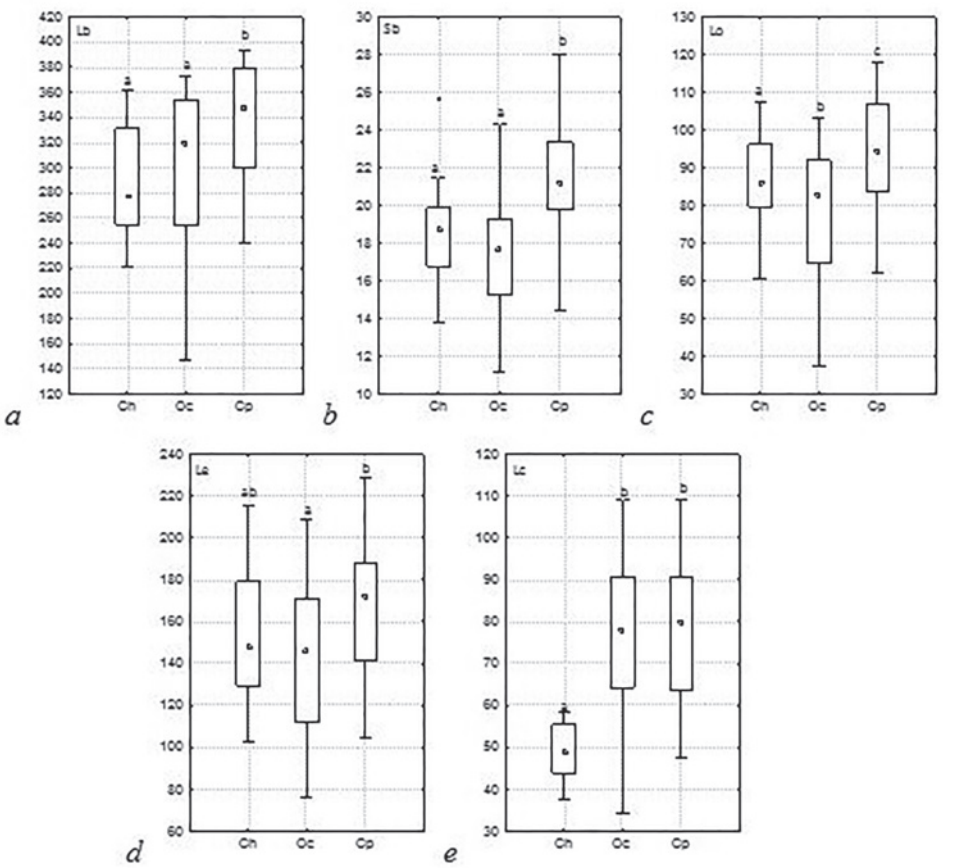

Fig. 2. Morphometric parameters of L_1 of *S. papillosus*: *a* — body length; *b* — body width; *c* — length of esophagus; *d* — length of intestine; *e* — length of tail; on the ordinate axis — length in μm, on the abscissa axis — species of host (Ch — *Capra hircus*, Oc — *Oryctolagus cuniculus*, Cp — *Cavia porcellus*).

No statistically significant difference was observed for tail length among the larvae from the feces of rabbits and guinea pigs (78–80 μm). However, statistically significant difference in this parameter was recorded for the larvae from the feces of goats and rabbits, and also of goats and guinea pigs (fig. 2, *e*).

The body length of L_2 from different species of hosts varied within 375–730 μm. The body length of these larvae from goats differed from the length of those from rabbits and guinea pigs. However, no differences in this parameter were observed between the larvae from rabbits and guinea pigs (fig. 3, *a*).

By contrast, width and length of the intestine statistically reliably differed between the larvae from rabbits and guinea pigs, and also between those from goats and guinea pigs. These indicators ranged within 16.2–34.1 and 176–396 μm respectively (fig. 3, *b*, *d*).

The length of the esophagus of *S. papillosus* larvae from different species of hosts equaled 67–192 μm, and the length of the tail was 43–224 μm. These parameters, similarly to the total body length, statistically differed between nematode larvae from goats and rabbits, and also from goats and guinea pigs. The difference in these two parameters in the first mentioned pair of host species equaled on average 15 and 40 μm, respectively, and in the second mentioned pair, 30 and 39 μm respectively (fig. 3, *c*, *e*). As with all previous measurements, the larvae from guinea pigs were the largest.

Infective third-stage larvae (L_3) have a thread-like shape (fig. 4, *a*), the esophagus occupies about a half of the body length, it has no dilatations or bulbs (fig. 4, *b*), the

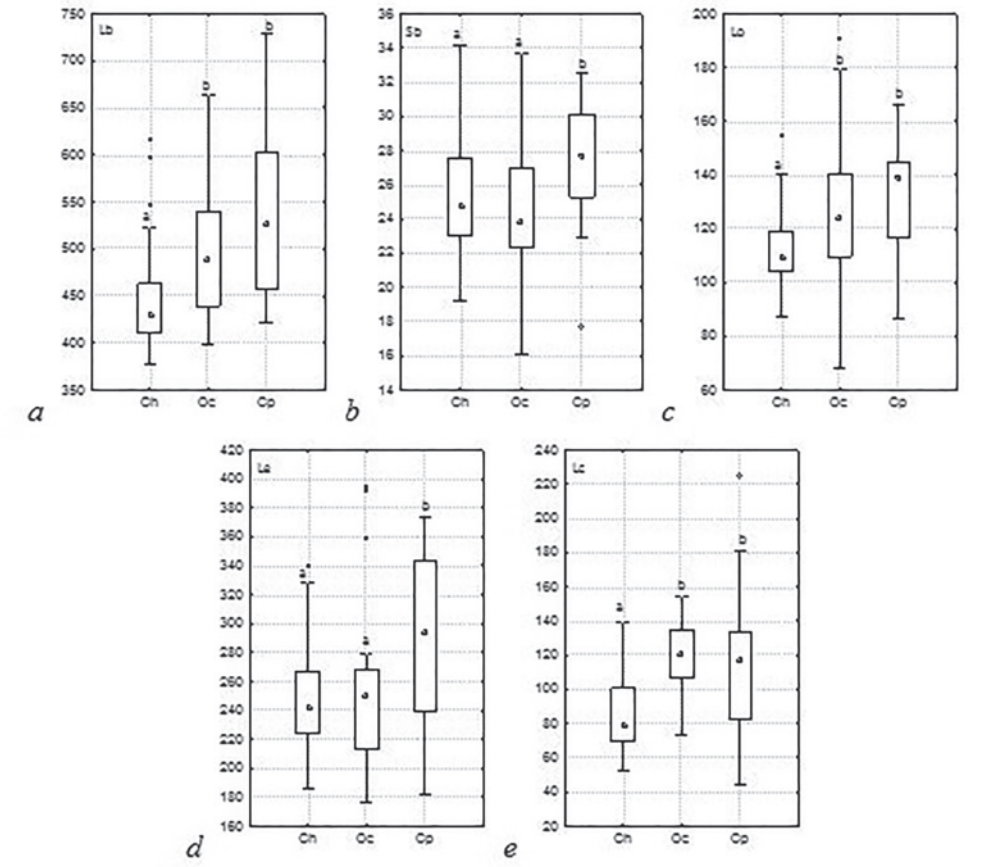

Fig. 3. Morphometric parameters of L_2 of *S. papillosus*: *a* — body length; *b* — body width; *c* — length of esophagus; *d* — length of intestine; *e* — length of the tail; on the ordinate axis — length in μm, on the abscissa axis – species of host (Ch — *Capra hircus*, Oc — *Oryctolagus cuniculus*, Cp — *Cavia porcellus*).

intestine is straight, filled with digestive granules, the tail is gradually tapering (fig. 4, *c*). The statistically reliable differences in morphology of L_3 from all species of hosts were recorded for the body width and the length of the tail (fig. 5, *b*, *e*). On average, among the larvae from goats, rabbits and guinea pigs, the first parameter equaled 20, 23 and 25 μm, and the second was 130, 140 and 110 μm, respectively.

The total body length of L_3 from all of the host species ranged from 383–825 μm, and the length of esophagus was 127–332 μm. Statistically significant differences were found in the two parameters among the larvae of this stage from goats and those from rabbits (fig. 5, *a*, *c*), and the second parameter was different between the larvae from goats and those from guinea pigs (fig. 5, *c*). No differences were observed in the length of intestine (fig. 5, *d*).

The males of *S. papillosus* have a cylindrical body (fig. 6, *a*) and two bulb-like dilatations in the esophagus (fig. 6, *b*), the gastrointestinal tract is filled with pigmented mass, and there are two spicules equal in shape and size (fig. 6, *c*, *d*).

The free-living females of *S. papillosus* have a cylindrical body, tapering towards the tail end (fig. 7, *a*). The esophagus has two dilatations (fig. 7, *b*), the vulva is located at the middle of the body length as a transverse slot. The uterus contained up to 7 eggs, some of them with larvae (fig. 7, *c*).

The body length of the mature free-living individuals of *S. papillosus* from different species of hosts ranged within 550–1000 μm on average, width — 25–45 μm, length of the esophagus — 110–210 μm, length of the intestine — 300–540 μm, length of the tail — 90–

Fig. 4. Third-stage larva of *S. papillosus*: *a* — general view; *b* — anterior part (the pointer indicates the place where the esophagus joins the intestine); *c* — posterior part; scale bars 100 μm.

210 μm (fig. 8, 9). There were statistically significant differences for all the morphometric parameters between *S. papillosus* males from rabbits and guinea pigs, and also between those from goats and rabbits (fig. 8).

Free-living females from the studied species of hosts had significant differences in the body length, and also in the length of the tail (fig. 9, *a*, *e*). Individually recorded statistically significant differences occurred between the size of the esophagus of females from rabbits

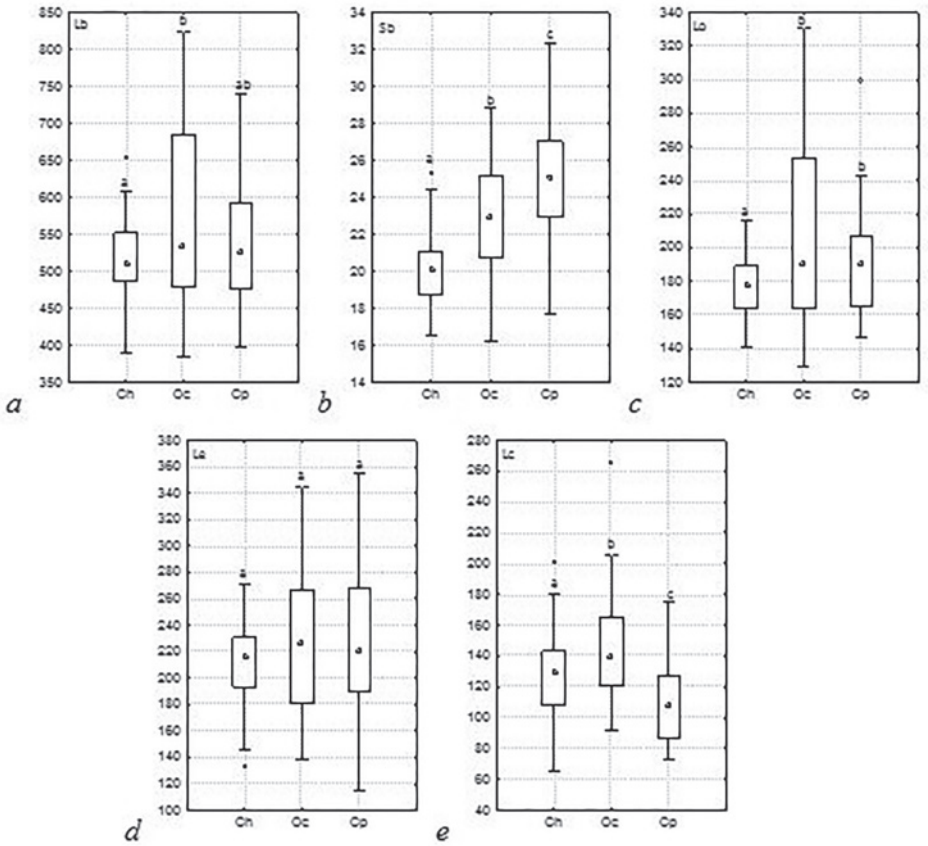

Fig. 5. Morphometric parameters of L_3: *a* — body length; *b* — body width; *c* — length of esophagus; *d* — length of intestine; *e* — length of the tail end; on the ordinate axis — length in μm, on the abscissa axis — species of host (Ch — *Capra hircus*, Oc — *Oryctolagus cuniculus*, Cp — *Cavia porcellus*).

Fig. 6. Mature male of *S. papillosus*: *a* — general view; *b* — anterior part (arrowheads indicate bulbs); *c* — caudal part with spicules in lateral view; *d* — caudal part with spicules in dorsal view; scale bars 100 μm.

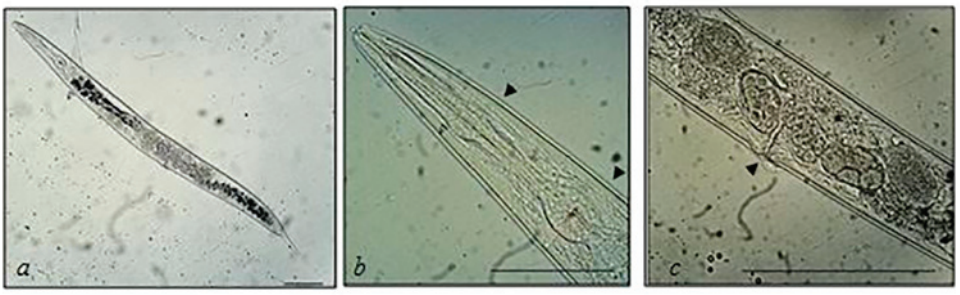

Fig. 7. Free-living female of *S. papillosus*: *a* — general view; *b* — anterior part (the arrowheads indicate the bulbs); *c* — uterus with eggs (the pointer indicates the genital opening); scale bars 100 μm.

and guinea pigs (fig. 9, *c*), the body width and the length of intestine of females from goats and guinea pigs (fig. 9, *b*, *d*). The distance from the anterior end to vulva, and also the number of eggs in the uteri of females were statistically different only between *S. papillosus* from goats and rabbits (fig. 9, *f*, *g*). Therefore, the maximum number of differences was observed between the *S. papillosus* males from goats and those from rabbits, and also between those from rabbits and those from guinea pigs (table 1).

Regarding all morphometric parameters, males of this nematode species statistically significantly differed depending on the species of hosts in the total length and width of the body, length of esophagus, intestine, tail end, body, spicules. At the same time, females from goats and rabbits were observed to show morphometric differences in all parameters: total body length and width, length of esophagus, intestine, tail, distance from anterior end to vulva, and also the number of eggs in the uteri.

Table 1. The results of morphometric analysis of three larva ages, males and females of *S. papillosus* from different hosts

Stage of larva development of sex of adult nematode	L_1			L_2			L_3			Male			Female		
Compared couple of the nematode's host	Ch-Oc	Oc-Cp	Ch-Cp	Ch-Oc	Oc-Cp	Ch-Cp	Ch-Oc	Oc-Cp	Ch-Cp	Ch-Oc	Oc-Cp	Ch-Cp	Ch-Oc	Oc-Cp	Ch-Cp
The number of statistically reliably differing parameters	2	4	4	3	2	5	4	2	3	6	6	1	7	3	4
Total number of the parameters compared	5			5			5			6			7		

Note. Ch — *Capra hircus*, Oc — *Oryctolagus cuniculus*, Cp — *Cavia porcellus.*

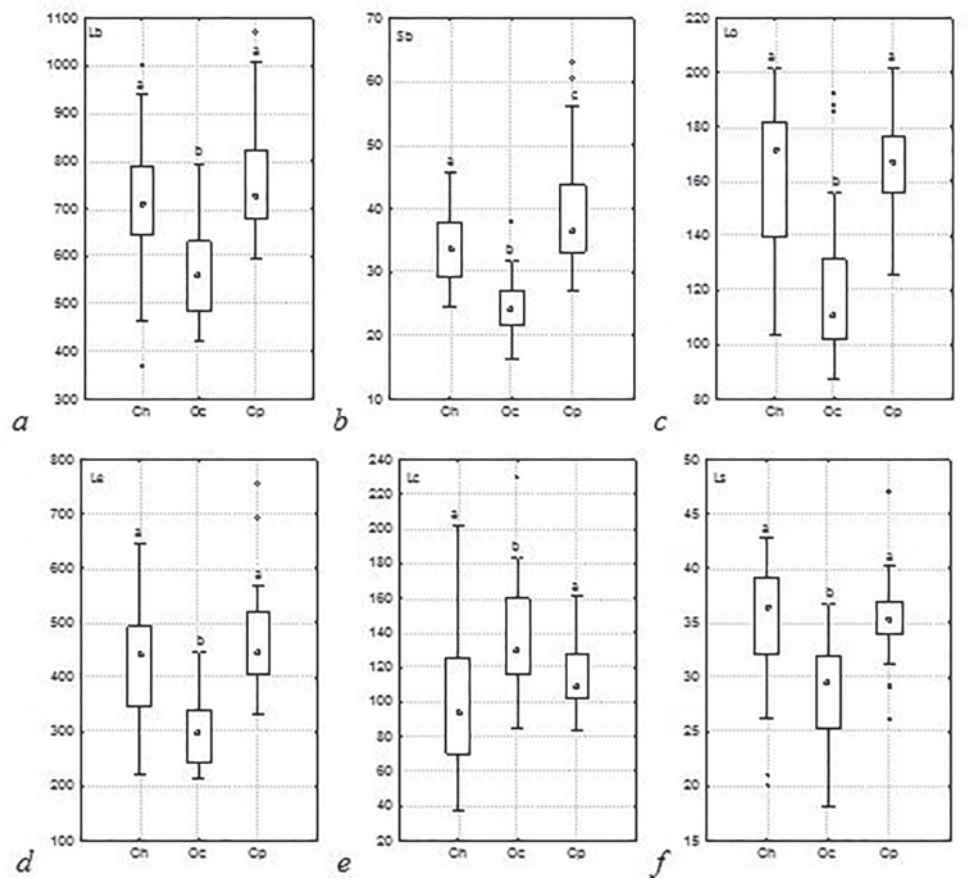

Fig. 8. Morphometric parameters of *S. papillosus* males: *a* — body length; *b* – body width; *c* — length of esophagus; *d* — length of intestine; *e* — length of the tail; *f* — length of spicules; on the ordinate axis — length in μm, on the abscissa axis — species of host (Ch — *Capra hircus*, Oc — *Oryctolagus cuniculus*, Cp — *Cavia porcellus*).

The species of the *Strongyloides* genus can infest a wide range of wild and domestic animals all around the world (Jacquiet et al., 1992; Sissay et al., 2007; Varodi et al., 2017). The most affected animals are young ones. The high extent of infestation often leads not only to decrease in the efficiency, and to susceptibility to other diseases, but also to the death of animals at an early age (Taira & Ura, 1991). Therefore, livestock farms suffer significant economic losses (Peter et al., 2015; Stachurska-Hagen et al., 2016; Thamsborg et al., 2017; Boyko & Brygadyrenko 2017, 2018). Data on the peculiarities of distribution, intensity of infestation, severity of the helminthiasis in cases of parasitization by *S. papillosus* in different species of hosts in relation to their age, geographical distribution, season and many other factors, as well as the morphometric features of nematodes of this genus are provided in a number of publications (Singh et al., 1997; Bekele, 2002; Agyei, 2003; Eysker et al., 2005; Jäger et al., 2005; Boyko et al., 2018).

Morphometric studies of various stages of development of *S. papillosus* were studied by scientists. Total body length of L_3, described by Basir (1950) at a morphometric study of *S. papillosus* obtained from sheep differs from our results. According to Basir (1950), the body length of the larvae of this stage is in the range 575–640 μm. This indicator is more than the size of *S. papillosus* obtained from goats, rabbits and guinea pigs 515, 530 and 525 μm, respectively. The total length of L_2 from sheep according Basir (1950) is 425–6004 μm on average. According to the results of our study, this indicator from goats, rabbits and guinea pigs is 425, 480, 525 μm on average. The length of L_1 from sheep (Basir, 1950) is in the range

of 235–400 μm. At L_1 from goats, rabbits and guinea pigs, this indicator is 280, 320, 350 μm on average. Total body length of free-living females (Basir, 1950) varies in the range 770–1110 μm. This indicator of *S. papillosus* from goats, rabbits and guinea pigs, according to the results of our study is 850, 1010 and 860 μm on average. The males according to Basir (1950) have a body length of 700–825 μm. Our results indicate some differences of

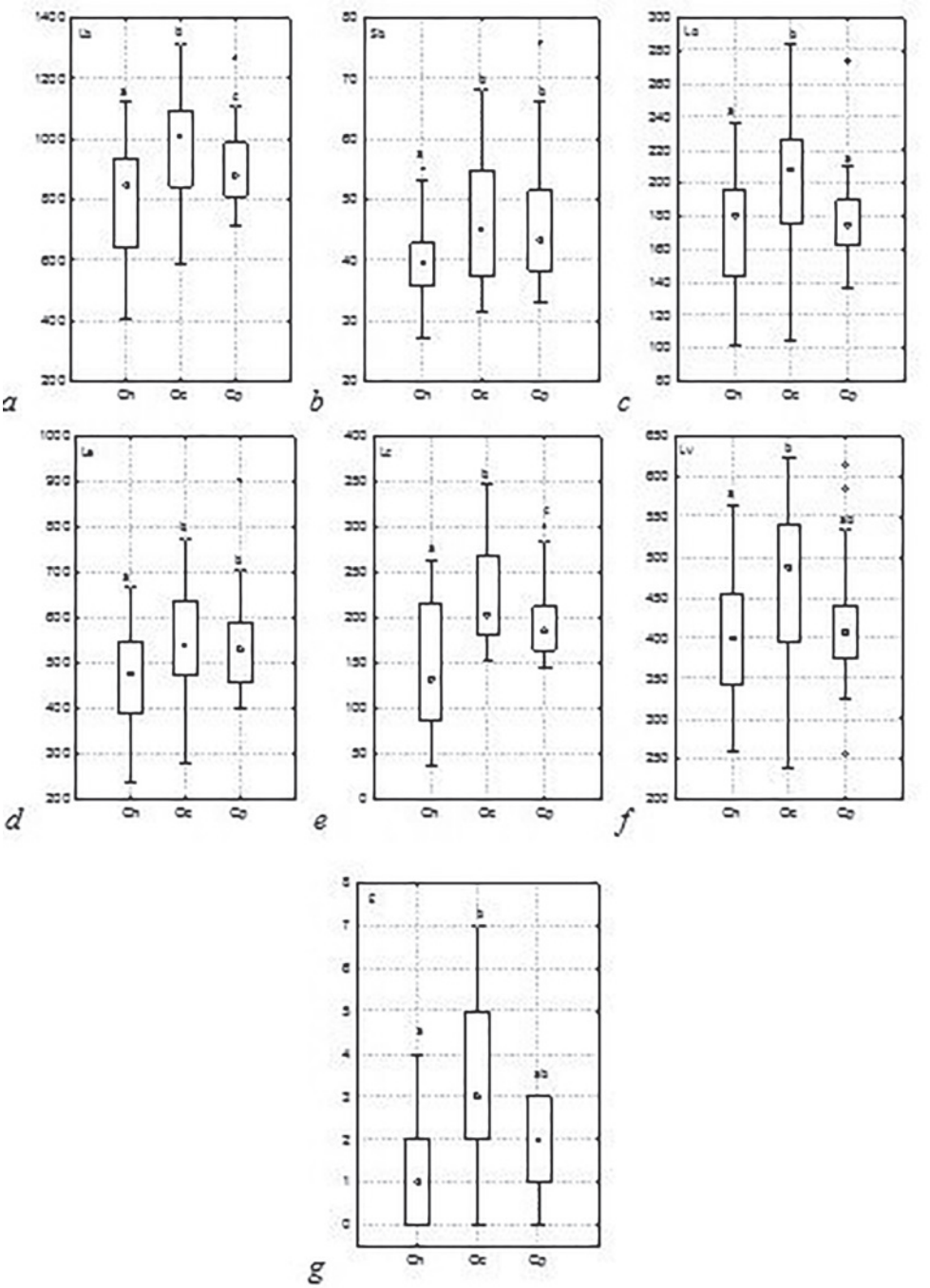

Fig. 9. Morphometric parameters of free-living females of *S. papillosus*: *a* — body length; *b* — body width; *c* — length of esophagus; *d* — length of intestine; *e* — length of tail; *f* — distance to vulva; *g* — number of eggs; on the ordinate axis — length in μm, on the abscissa axis — species of host (Ch — *Capra hircus*, Oc — *Oryctolagus cuniculus*, Cp — *Cavia porcellus*).

S. papillosus from rabbits. The body length of males is 550 μm. Males from goats and guinea pigs didn't exceed of 710 and 720 μm on average. According to Basir (1950), the length of the esophagus of males from sheep is 130 μm on average. The results of our studies show some difference in the length of the esophagus of males from goats and guinea pigs (165, 170 μm on average). This indicator of free-living females from sheep is more by 10 μm (Basir, 1950). According to the results of our studies, the length of the esophagus of free-living females significantly longer and averages 180, 210, and 170 μm from goats, rabbits and guinea pigs.

Dyomkina (2006) studied the morphological features of *S. papillosus* from cattle. Rabditiform larvae of this species of nematodes from cattle had a body length of 368–619 μm (514 μm on average). According to the analysis of our results, L_1 from goats, rabbits and guinea pigs is significantly less. Their length does not exceed 350 μm on average. The total body length of the L_2 of these nematodes from cattle, goats, rabbits and guinea pigs is not significantly different. This indicator of L_3 from goats, rabbits and guinea pigs does not differ from the results obtained by Dyomkina (2006), which varies between 502–686 μm. The body length of male from cattle is significantly longer than from goats, rabbits and guinea pigs and averages 825 μm. This indicator of free-living females from cattle varies between 770–1172 μm (1008 μm on average) (Dyomkina, 2006). Free-living females from goats and guinea pigs are significantly smaller. According to the results of our research, this indicator is lower by 158 and 148 μm on average.

Van Wyk et al. (2004) indicates the ratio of the length of the esophagus to the total body length of L_3 from small ruminants and cattle. According to results of morphometry, the esophagus occupies about 40 % of the total body length of the larvae, which is slightly different from the results of our morphometric studies of *S. papillosus* from goats, rabbits and guinea pigs (36 % on average). The total body length of L_3 from small ruminants and cattle is 600 μm on average (Van Wyk et al., 2013). This indicator is also slightly higher than that obtained by Dyomkina (2006) and our research.

Most authors consider all *Strongyloides* of agricultural Ruminantia to be the same species — *S. papillosus*. According to Eberhardt et al. (2008), most *S. papillosus* found in cattle and sheep belong to separate, genetically isolated populations. Such research was conducted in Southern Germany (Eberhardt et al., 2008). Our studies on intraspecific variability in the morphometric parameters of *S. papillosus* in different species of hosts indicate statistically significant morphometric differences; changes in the body length and width, length of esophagus, intestine, tail end of *S. papillosus* at different stages of development, in the spicules of males, length from vulva to mouth, and also the number of the developed eggs in the womb cavity of free-living females.

Conclusion

As a result of the experiment, we determined statistically significant differences in morphometric parameters between larval stages and mature individuals (free-living males and females) of *S. papillosus* originating from in different species of hosts (goats, rabbits and guinea pigs). In comparison of body length and width, length of esophagus, intestine, tail, spicules, the highest number of differences was found between the males of *S. papillosus* from goats and rabbits, and also those from rabbits and guinea pigs.

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