Morphometric parameters of egg-shells and third-stage juveniles as diagnostic features of *Oesophagostomum dentatum* and *O. quadrispinulatum* (Strongyloidea: Chabertiidae)

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Accepted for publication 25 May 2020

Summary. The diagnostic importance of morphometric data of egg-shells and infective third-stage juveniles (J3) was studied for nodular worms of pigs, *Oesophagostomum dentatum* and *O. quadrispinulatum* (Nematoda: Strongyloidea). A significant difference was found between the species in the lengths of the tail filament of third-stage juveniles. When expressed as the ratio of the tail filament length to the total length of J3, values were 14.65 ± 0.41% for *O. dentatum* and 16.46 ± 0.44% for *O. quadrispinulatum*. By contrast, egg lengths of these two species did not differ significantly when measured shortly after evacuation from a host intestine.
Key words: identification, nodular worms, pig intestinal parasites, size of eggs.

Oesophagostomum dentatum (Rudolphi, 1803) and *O. quadrispinulatum* (Marcone, 1901) are common intestinal parasites of wild and domesticated pigs. The host range for representatives of the genus *Oesophagostomum* Molin, 1861 includes different mammals (small ruminants and cattle, non-human primates); additionally, in some tropical areas these nematodes were found parasitic in a human intestine. The parasitism by *Oesophagostomum* (oesophagostomiasis) results in the formation of nodules on the intestinal walls, which is a reason for the common name of these nematodes, *i.e.* nodular worms. Although industrialised pig farms are usually free of *Oesophagostomum* species (Roepstorff *et al.*, 2011), smaller herds, both free-range and kept indoor, are often heavily infected with these parasites (Nosal *et al.*, 2008). *Oesophagostomum quadrispinulatum* is considered to be more pathogenic than *O. dentatum* (Christensen *et al.*, 1997; Nosal *et al.*, 2007) and it was also reported that *O. quadrispinulatum* has a higher tolerance than *O. dentatum* to anthelmintic treatments (Varady *et al.*, 1996). There are four stages of the *Oesophagostomum* life cycle present in the environment: eggs and the first three juvenile stages, J1, J2 and infective J3. Though, the morphology of eggs and J3 is generally close in *Oesophagostomum* species parasitic in swine (Zajac & Conboy, 2012; Ahiabor & Lawson, 2015), the data reported by Maplestone (1930) for the size of eggs of *O. dentatum* and *O. quadrispinulatum* and Van Wyk & Mayhew (2013) for the size of J3 of several other species might point at their potential as diagnostic features. The goal of this study was to develop the method of discrimination between *O. dentatum* and *O. quadrispinulatum* based on morphometric parameters of eggs and J3 of these nematodes for pig farms in our region.

MATERIAL AND METHODS

The nematodes were obtained after autopsy of 6-24 month-old pigs in the slaughterhouse of the 'Dubkovsky Sausages' company (Republic of Crimea) in 2016-2018. Mature females of the genus *Oesophagostomum* were obtained from the large colon of slaughtered animals. These females were identified to the species level using morphological characters according to Dakova & Panayotova-

Pencheva (2017). Morphological identification was confirmed by the sequence analysis of *Cox*I mtDNA and ITS2 rDNA performed by O.P. Maluchenko (All-Russian Research Institute of Agricultural Biotechnology).

For extraction of nematode eggs, faeces of these pigs were also collected. The *Oesophagostomum* eggs were extracted from faecal samples by the flotation method in a saturated solution of ammonium nitrate (Saphiullin, 2001). Eggs were also obtained directly from the gonads of gravid *Oesophagostomum* females. All of the eggs were collected from the pigs bred in the same part of the Crimean peninsula during summer to avoid seasonal variations in egg morphometrics observed previously (Pasechnik *et al.*, 2020). To obtain J3, nematode eggs were cultivated at least 14 days in the faeces and identified under a light microscope at low (×100) and high (×400) magnification after their removal to water, sedimentation for several hours and fixation with Lugol solution (Saphiullin, 2001). The eggs were divided into two groups: one consisting of eggs at the pre-segmentation stage of development (zygote, no cleavage), and another of eggs at the early segmentation stage (4-8 blastomeres). To estimate the size of J3, four parameters were obtained: L – J3 body length with the cuticular sheath; F – length of the tail filament; T – a total J3 body length with the filament and W – body width (Fig. 1).

The morphometrics of eggs and J3 were obtained using the high resolution images (3968 × 2976 pixels) taken at the low magnification of the light microscope (×100) with the ImageJ computer program (National Institutes of Health, Washington, USA). The dimensions were automatically recalculated from pixels to micrometres with the calibration Set Scale value of 3.8 px μm^{-1}.

The Student's t-test was used to estimate the significance of differences between mean values of the data obtained, as Shapiro-Wilk test proved that these were normally distributed.

RESULTS

Morphometric parameters of *O. dentatum* and *O. quadrispinulatum* eggs. At the pre-segmentation stage *in uteri*, the egg-shell length was similar in both species: 60.5 ± 0.6 and 61.8 ± 0.9 µm in *O. quadrispinulatum* and *O. dentatum*, respectively. At the same stage, the egg-shells of *O. quadrispinulatum* were wider than those of *O. dentatum* by *ca* 7.04%. At the early segmentation stage, egg-shells of *O. quadrispinulatum* were longer than those of *O. dentatum* by *ca* 4.8% (Table 1).

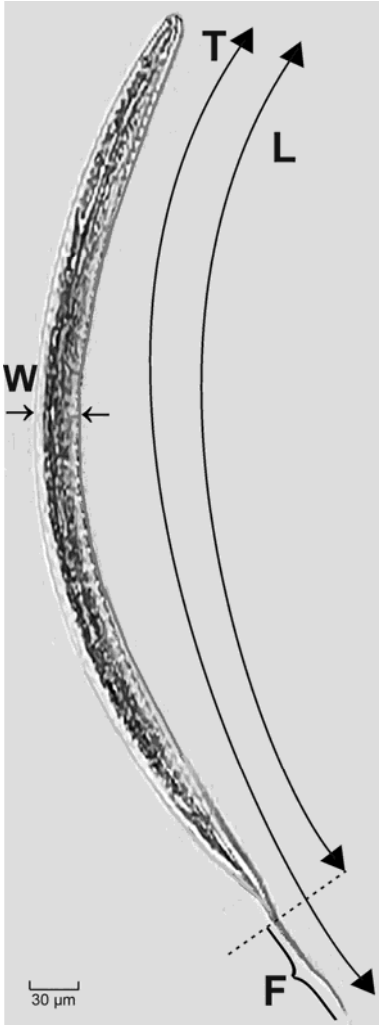

Fig. 1. Third-stage juvenile (J3) of *Oesophagostomum dentatum*. L – J3 body length with the cuticular sheath; F – length of the tail filament; T – a total J3 body length with the filament; W – body width. Scale bar = 30 µm.

Egg-shell sizes measured during the first 24 h in the environment after completion of the flotation were nearly identical: 68.0 ± 0.8 × 37.9 ± 0.4 for *O. dentatum* and 68.9 ± 0.9 × 38.2 ± 0.5 µm for *O. quadrispinulatum* (Table 1).

It was observed that during the development *in uteri*, the egg-shell length in *O. dentatum* increased by 10.08% and in *O. quadrispinulatum*, by 17.67%. For the latter species, a significant decrease of egg-shell length by 3.34% was observed for the eggs on the first day in the environment (Table 1).

Morphometric parameters of J3. The duration of the juvenile development of *O. dentatum* and *O. quadrispinulatum* in our study was different. The J3 of *O. quadrispinulatum* were observed in pig excrements on the 12th day while those of *O. dentatum* on the 22nd day only.

Table 1. Measurements of *Oesophagostomum dentatum* and *O. quadrispinulatum* eggs.

Stage of development	*O. dentatum*			*O. quadrispinulatum*		
	Number of measured eggs (n)	Size of eggs (mean ± s.d.), µm		Number of measured eggs (n)	Size of eggs (mean ± s.d.), µm	
		length	width		length	width
Pre-segmentation	317	61.8 ± 0.9	36.6 ± 0.7	322	60.5 ± 0.6	39.2 ± 0.6(**)
Early segmentation	330	67.9 ± 0.6*	38.4 ± 0.6	331	71.2 ± 0.6*(**)	39.8 ± 0.3
In the faeces (first day in the environment)	318	68.0 ± 0.8*	37.9 ± 0.4	216	68.9 ± 0.9*	38.2 ± 0.5

* – the differences are statistically significant being compared with the pre-segmentation eggs ($P \leq 0.01$).
(**) – the differences are statistically significant being compared with the *O. dentatum* eggs ($P \leq 0.01$).

Table 2. Morphometric parameters of third-stage juveniles of *Oesophagostomum dentatum* and *O. quadrispinulatum*.

Nematode species	Number of juveniles measured (n)	Third-stage juveniles' length and width (mean ± s.d.), µm				
		L, body length with the cuticular sheath	F, length of the tail filament		T, total length with the filament	W, body width
		µm	µm	%		
O. dentatum	125	471.7 ± 4.3	81.2 ± 2.8	14.65 ± 0.41	552.9 ± 5.7	29.9 ± 0.5
O. quadrispinulatum	115	550.9 ± 7.1*	107.5 ± 3.6*	16.46 ± 0.44*	658.5 ± 8.7*	36.2 ± 0.5*

* – the differences are statistically significant being compared with the sizes *O. dentatum* ($P \leq 0.01$).

The length of the tail filament of J3 of *O. quadrispinulatum* is significantly longer than that of *O. dentatum* (1.3×) (Table 2): 107.5 ± 3.6 *vs* 81.2 ± 2.8 µm, respectively. In *O. quadrispinulatum*, the average body length (L) of a juvenile with the cuticular sheath is significantly longer than that in *O. dentatum*: 550.9 ± 7.1 and 471.7 ± 4.3 µm, respectively. The average total length with the filament (T) of *O. quadrispinulatum* accordingly exceeds that of *O. dentatum*: 658.5 ± 8.7 and 552.9 ± 5.7 µm, respectively. The J3 of the former species also have greater body diameters than that of the latter species: 36.2 ± 0.5 *vs* 29.9 ± 0.5 µm (Table 2).

DISCUSSION

Considerable variations of an egg-shell size were reported for the species of *Oesophagostomum*. For *O. dentatum*, the reported range of an egg-shell size is 60-80 × 35-45 µm (Maplestone, 1930; Cherepanov *et al.*, 1999; Nosal *et al.*, 2013) and for *O. quadrispinulatum*, 48-63 × 28-33 µm (Maplestone, 1930; Nosal *et al.*, 2013). The ranges overlap and, therefore, are not statistically significant. The measurements of egg-shells of *O. dentatum* in our material were consistent with the previously reported data while those for *O. quadrispinulatum* exceeded that reported by Maplestone (1930) but corresponded to those of Nosal *et al.* (2013), apart from the smaller egg-shell diameter. Our data demonstrated that the morphometric parameters of *Oesophagostomum* eggs changed in the course of development (Table 1). For the eggs excised from the uteri of gravid females, the differences in the lengths at the early segmentation and the width at the pre-segmentation stage between *O. dentatum* and *O. quadrispinulatum* were observed.

In our study, eggs of both species obtained from faeces and measured after 24 h in the environment did not differ in size significantly, thus preventing using the parameter for differentiation. Such observation contradicts the suggestion by Maplestone (1930) to use the measurements of egg-shells to distinguish between *O. dentatum* and *O. quadrispinulatum*. It seems that only measurements of eggs *ex uteri* can be used reliably for discrimination of these two species.

The analysis of morphometrics of J3 has shown a considerable difference in the ratio of the tail filament length to the total length of J3 between these two species of *Oesophagostomum* (14.65 ± 0.41% for *O. dentatum vs* 16.46 ± 0.44% for *O. quadrispinulatum*) (Table 2).

Thus, based on our study, we can conclude that the parameters of a length and width of egg-shells

from the uteri of gravid females and the ratios of the tail filament length to the total length of J3 can be used for the species discrimination of the Crimean populations of the two common pig nodular worms, *O. dentatum* and *O. quadrispinulatum*.

ACKNOWLEDGEMENTS

We are sincerely thankful to T.V. Kerimov, the director of the firm 'Dubkovsky Sausages', for the opportunity to use the technical base of the production laboratory of the enterprise to conduct our research; we are also grateful to Dr O.P. Maluchenko and staff people of the All-Russian Research Institute of Agricultural Biotechnology for the PCR-confirmation of the identification of nematode species.

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Г.А. Лукьянова, Л.В. Ягенич и А.А. Пасечник. Морфометрические параметры яиц и личинок третьей стадии как диагностические признаки *Oesophagostomum dentatum* и *O. quadrispinulatum* (Strongyloidea: Chabertiidae).

Резюме. Изучена диагностическая значимость морфометрических параметров яиц и инвазионных личинок третьей стадии эзофагостом свиней, *Oesophagostomum dentatum* и *O. quadrispinulatum* (Nematoda: Strongyloidea). Достоверная разница была обнаружена в длине хвостовой нити личинок третьей стадии (J3) обоих видов, выраженная отношением длины хвостовой нити к общей длине личинки J3. У *O. dentatum* она составила 14.65 ± 0.41% и 16.46 ± 0.44% – у *O. quadrispinulatum*. Размеры яиц этих двух видов в свежих фекалиях достоверно не отличалась.