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

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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 Cox1 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).

**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.

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>O. dentatum</th>
<th>O. quadrispinulatum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of measured eggs (n)</td>
<td>Size of eggs (mean ± s.d.), μm</td>
</tr>
<tr>
<td></td>
<td>length</td>
<td>width</td>
</tr>
<tr>
<td>Pre-segmentation</td>
<td>317</td>
<td>61.8 ± 0.9</td>
</tr>
<tr>
<td>Early segmentation</td>
<td>330</td>
<td>67.9 ± 0.6*</td>
</tr>
<tr>
<td>In the faeces (first day in the environment)</td>
<td>318</td>
<td>68.0 ± 0.8*</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Nematode species</th>
<th>Number of juveniles measured (n)</th>
<th>L, length with the cuticular sheath</th>
<th>F, length of the tail filament</th>
<th>T, total length with the filament</th>
<th>W, body width</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. dentatum</td>
<td>125</td>
<td>471.7 ± 4.3</td>
<td>81.2 ± 2.8</td>
<td>14.65 ± 0.41</td>
<td>552.9 ± 5.7</td>
</tr>
<tr>
<td>O. quadrispinulatum</td>
<td>115</td>
<td>550.9 ± 7.1*</td>
<td>107.5 ± 3.6*</td>
<td>16.46 ± 0.44*</td>
<td>658.5 ± 8.7*</td>
</tr>
</tbody>
</table>

* – the differences are statistically significant being compared with the sizes O. dentatum (P ≤ 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*.

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**REFERENCES**


