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ON *TAENIA HYDATIGENA* (PALLAS, 1766) BIOLOGICAL CHARACTERISTICS, IMPORTANT FOR THE TENUICOL CYSTICERCOSIS EPIZOOTOLOGY

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Abstract

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Medium and high experimental infestation with the species *Taenia hydatigena* was carried out on dogs. The duration of the parasite prepatent period was measured for both infestations. The strong motility and spontaneous liberation of the gravid segments were confirmed. The average daily number of released proglottids was established as well as their number per one specimen of *T. hydatigena* during the first 15 weeks of the patent period. The average daily number varied within 6 – 26 proglottids and the number per specimen of the tapeworm – 0.67 and 2.89.

A linear tendency of the mature segments release was found during the period of the study. The maximum release during the experimental infestation was recorded in the 10th and 12th week of patency.

The morphology of the uterus in gravid proglottids during the same 15-week period was also studied. It was established that the uterus of the gravid segments was completely filled with taeniid eggs at the beginning of the 13th week of patency.

Key words: Taenia hydatigena, biological characteristics, epizootology, tenuicol cysticercosis, gravid proglottids

Introduction

The biology of species from genus Taenia has been the object of study of parasitologists for many years. Scientists have shown a special interest in their characteristics such as type and number of hosts, duration of the prepatent period and longevity of the adults, number and dynamics of the gravid proglottids release. All those biological characteristics are significant for the epizootology and diagnosis of taenioses and their larval cestodoses in a number of animal species (Soulsby, 1968; Georgieva and Kamenov, 1993; Kamburov et al., 1994; Taylor et al., 2007).

Taenia hydatigena (Cestoda: Taeniidae) is one of the earliest described species of the family Taeniidae, currently found worldwide. This cestode develops in domestic and wild canids, bear and rarely in felids that are definitive hosts (Kamburov et al., 1994; Samuel et al., 2001; Kamenov et al., 2009). The parasite does not reach sexual maturity in felids (Rausch et al., 1983) therefore; it does not play an important role in the epizootology of the larval cestodosis. Domestic and wild ruminants, swine, equine species and, more rarely, about 50 other mammal species are the intermediate hosts, in which the larvae of type cysticercus (*Cysticercus tenuicollis*) occurs as a parasite. *T. hydatigena* is the most common taeniid species in domestic dogs in Bulgaria and in Europe and its larvae cause a severe and sometimes lethal cestodosis (called tenuicol cysticercosis) in lambs, goat kids and piglets in cases of heavy infestation (Kanchev, 2013). One of the major reasons for the wide distribution of the species is the fact that the representative is characterized with high productivity of sexual products, i.e. gravid segments, the eggs in which reach the quantity of 31000 – 38000 per proglottid (Coman and Rickard, 1975; Deplazes and Eckert, 1988). The average daily number of released proglottids per specimen of *T. hydatigena* varies from 0.70 (Sweatman and Plummer, 1957), 0.75 – 1 (He, 1983; Kamburov et al., 1994), 0.36 – 2.28 (Featherston, 1969) and 2.30-4.90, according to Deplazes and Eckert (1988). The result is a daily average of 100 000 eggs released from a mature tapeworm (Gregory, 1976).

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According to a number of authors, the duration of the period to reaching sexual maturity in *Taenia hydatigena* is as follows: 42 to 79 days in primary infestation and 70 – 100 days in re-infestation. The patent period of *T. hydatigena* varies within 41 - 342 days and rarely 860 days in primary infestation (Sweatman and Plummer, 1957; Featherston, 1969; Gradinarski, 1987; Deplazes and Eckert, 1988; Kamburov et al., 1994; Fisher and McGarry, 2006; Taylor et al., 2007). According to a number of leading authors, the indexes of species and age of the definitive host do not have a significant effect on the duration of the patent period but it is considerably shorter in cases of re-infestation (Gregory, 1976; Deplazes and Eckert, 1988).

Goals and Tasks

The goal of the present research was to study some important characteristics of the biology of *Taenia hydatigena* in experimental infestation.

To achieve our goals, we set the following tasks:

- To perform a double experimental infestation of a typical definitive host to study the development of *Taenia hydatigena* in different infestation rates
- To study the duration of the prepatent period in case of medium and heavy infestation
- To study the specifics and dynamics of gravid proglottids release during the first 15 weeks of the patent period
- To examine the morphology of gravid segments during the same 15-week period

Materials and Methods

Experimental animals

We used the typical definitive hosts as experimental animals – dogs, two animals at the age of two months, without breed, grown in the premises for experimental animals from birth, non-immune to the parasite and free from other infestations. The dogs were vaccinated by the standard scheme and treated for helminths twice. The hosts were grown in conditions that excluded spontaneous infestation and complied with the requirements of humane treatment of experimental animals (BG Regulation № 20, 2012). The cysticerci for the infestation (infective *Cysticercus tenuicollis*) were retrieved during a hygienic assessment after a regular sheep slaughter in a licensed Bulgarian slaughter house. One of the dogs was infected with 14 cysticerci and the other - with 5.

Helminthoscopy method

We detected and collected the gravid proglottids from both dogs after the prepatent period by the Helminthoscopy method. Using the same method, we collected mature *Taenia hydatigena* specimens after preliminary treatment of dogs, with 1‰ water solution of Arecolinum hydrobromicum in a dose of 3 mg/kg body mass. We fixed and conserved the segments and specimens in 70% ethanol.

Compressorium method

The research on the morphology of the gravid segments was done by the compressorium method after clearing procedure in 50% glycerol, described by Kamenov and Radev (2002). The microscopic research was done with the available equipment at the Faculty Laboratory of "Parasitology and parasitic diseases of animals" at the University of Forestry - Sofia.

The statistical analysis

The statistical analysis of the experimental results was done with the software Prism® and Ky Plot® by the following algorithm:

- ✓ The primary data were checked for normal Gaussian distribution. The Kolmogorov-Smirnov tests showed that data were normally distributed.
- ✓ Grub's test was used to check for extreme values, which were not found. This was enough to make a further statistical analysis of the data.

Results and Discussion

The duration of the prepatent period was evaluated for two experimental infestations in host domestic dogs:

- prepatent period in infestation with 5 larvae – 47 days.
- prepatent period in infestation with 14 larvae – 69 days.

According to our opinion, the difference in the duration of the prepatent period in the definitive hosts of identic species and age was due to the different number of taenias that were developing in the small intestine simultaneously. When the number of the developing cestodes was smaller, they reached sexual maturity faster and started releasing proglottids. The duration of the prepatent period in the experimental infestation of dogs was within the ranges described by most authors (Sweatman and Plummer, 1957; Featherston, 1969; Deplazes and Eckert, 1988; Kamburov et al., 1994; Fisher and McGarry, 2006; Taylor et al., 2007). It was with 5 days longer than the minimum for the species, as described in the PhD thesis of Gradinarski (1987).

The number of specimens of *T. hydatigena* species that had reached maturity was counted after the end of the experiments and they were 5 for the case of infestation with 5 larvae and 9 for infestation with 14 cysticerci. In the case of the infestation with a smaller number of cysticerci (5), all

larvae developed into adult taenias, while in the infestation with a larger number, only 65% did. This was probably due to the competition between developing cestodes for the vital resources of the host and the release of a different quantity of metabolic products that lead to a longer period of growth or inhibited the process of reaching sexual maturity in the case of heavy infestation.

Our study confirmed the findings of Bulgarian and foreign authors about the biological characteristics of *T. hydatigena*, namely, the gravid segments detached from the strobila separately and were highly movable (Deplazes and Eckert, 1988; Kamburov et al., 1994; Taylor et al., 2007). The proglottids detached from *T. hydatigena* strobila could possibly leave the anal opening of final hosts spontaneously and stick to the skin or hair in the perianal area. In our case, this phenomenon was observed right before or after the defecation of the experimental dogs.

Our data on the dynamics of the release of gravid *T. hydatigena* proglottids during the 15 weeks from the beginning of the patent period are given in Table 1.

The data in Table 1 clearly show that the daily number of released proglottids throughout the 15-week period varied within 2 – 45. The second column of the table shows the data on the average daily number of released segments that started from 9 ± 2 and reached a maximum of 21 ± 5. Table 1 also represents the value of the average daily number of proglottids per specimen of *T. hydatigena* – from 0.67 to 2.89 segments during the weeks.

Our results on the average daily number of released proglottids as well as number per specimen support the publications of Sweatman and Plummer (1957) and He (1983), their data equaling the lower level of our data. The values of the indexes in our study were most close to those of Featherston (1969) and lower than those of Deplazes and Eckert (1988) with regard to the indexes of number of proglottids daily and per specimen.

The analysis of the data showed a tendency to a gradual increase of the number of released gravid segments (Table 1, column 2) that was maintained up to the 12th week of patency. A larger number of released segments vs. the average were observed during the 6th, 9th and 11th weeks and the largest - in the 10th and 12th weeks. After the 12th week, the number of *T. hydatigena* proglottids in the faeces dropped to the steady 1.5 per available mature specimen. Another observation showed that the release of one or less gravid segments daily per specimen of the taenia occurred only up to the 8th week of the patency with one exception in week 6 (Table 1., column 4). After the 9th week, an average of more than one segment per available specimen was released from the strobila of *T. hydatigena*. The maximum of release was reached

Table 1
Data on the release of gravid proglottids during the first 15 weeks of the patent period in experimental infestation of dogs with 9 *Taenia hydatigena* specimens

Week of patency	Daily number of released gravid proglottids (min - max)	Average daily number of gravid proglottids $\overline{X}$±SEM	Average daily number of gravid proglottids per specimen of *T. hydatigena* (min - max)
	2-15	9±2	0.78 – 1.22
	4-15	10±2	0.89 – 1.33
	3-17	9±2	0.78 – 1.22
	4-18	10±2	0.89 – 1.33
	2-19	9±3	0.67 – 1.33
	4-26	14±3	1.22 – 1.89
	5-22	10±2	0.89 – 1.33
	2-17	10±2	0.89 – 1.33
	4-29	13±3	1.11 – 1.78
	7-31	18±3	1.67 – 2.33
	6-28	14±3	1.22 – 1.89
	7-45	21±5	1.78 – 2.89
	5-25	12±3	1.00 – 1.67
	2-27	12±3	1.00 – 1.67
	3-19	14±2	1.33 – 1.78

in weeks 10th and 12th, i.e. more than two proglottids per taenia daily. The established tendency in the release of proglottids that contained respectively infective taeniid eggs is very important for the epizootology of tenuicol cysticercosis. This biological characteristic of *T. hydatigena* caused different levels of environmental contamination with infective eggs during the separate weeks of the patency. The presence of a large number of eggs on pastures carries a potential risk of parasitic infestation in the intermediate hosts.

The tendency of the number of released gravid proglottids during the first 15 weeks of the patent period is shown on Figure 1.

The graphics on Figure 1 clearly shows the ideal linear tendency in the release of gravid *T. hydatigena* proglottids for the period of study. The same figure shows the confidence interval (the dotted lines), found to be small, therefore, the linear character of the curve was significant. The linear increase of the release is categorically confirmed by the following statistical indexes: P = 0.0033, correlation coefficient (as per Pearson) r = 0.6422 and slope of the straight line S = 0.5168 ± 0.1716.

The statistical processing of the data definitely showed the gradual increase of the proglottid productivity of *T. hydatigena* with two peaks, observed in the 10th and 12th weeks of patency. After the peak of release (week 12), the number of segments decreased and maintained a steady level of 1.5 proglottids per mature specimen daily. The weeks with peak proglottid release carried the highest risk of environmental contamination with infective eggs.

In our morphological studies, we found that the uterus of the released proglottids was full with a very small number of taeniid eggs during the first weeks of the 15-week period. Microscopically, we established the following feature: primarily, the zones with taeniid eggs in the uterus were small and disseminated in the gravid segment. This specificity of the released proglottids of *T. hydatigena* was maintained up to the 8th week of patency (Figure 2). A considerable increase of the taeniid eggs was observed in the released segments between weeks 8 and 12 (Figures 3 and 4). The uterus of the gravid proglottids was completely packed with eggs in week 13 (Figure 5.) as well as during the remaining weeks to week 15.

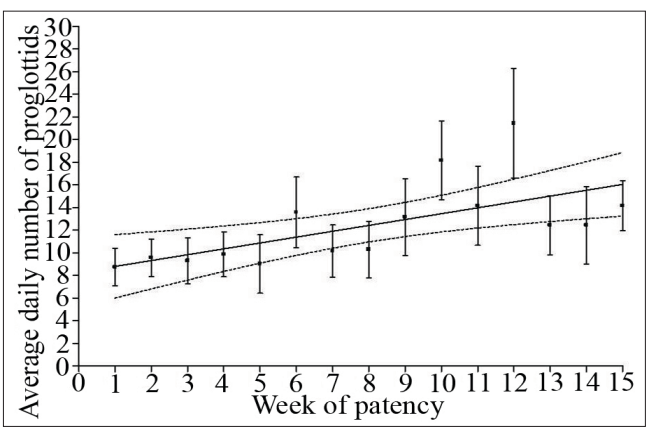

Fig. 1. The dynamics of gravid proglottids release in experimental infestation in dogs with 9 *T. hydatigena* specimens

The presence of a different number of taeniid eggs in the uterus of *T. hydatigena* gravid proglottids in different parts of the patent period is also an important feature for the epizootology of tenuicol cysticercosis because of the possibility for infestation of intermediate hosts. It is important to know exactly in which period of patency segments have the largest number of taeniid eggs and carry the highest risk of infestation. Most of the lethal infestations in lambs and goat kids resulted from the intake of one or more whole gravid segments with the grass feed (Kanchev, 2013). Some authors explain this very important biological characteristic of *T. hydatigena* with the fact that most of the eggs are released still in the intestinal tract of the dog from proglottids that were already detached from the strobila. It has been experimentally proven that in the same dog, the segments still attached to the end of the strobila have a uterus, full of eggs, unlike those that have detached recently (Samuel et al., 2001). Most probably, this was caused by the strong motility of the proglottids after detachment, most of the eggs being released in the intestine of the definitive host during the segment strong muscular contractions. This biological feature of *T. hydatigena*, however, was not observed in the segments, detached during and after week 13 of patency. This was proved from the microscopic observation – at that time, the uterus was packed with taeniid eggs. Our present studies also proved that the ingestion of whole gravid segments carried the highest risk for infecting domestic and wild animals after week 12 of patency.

There were no studies on the biological characteristics of *T. hydatigena*, important for the epizootology, in Bulgaria until the present moment. We didn't find such detailed characteristics of the dynamics of proglottid release in foreign sources, or complete data on the morphology of the gravid uterus during the different weeks of patency.

Conclusions

- The duration of the prepatent period of *Taenia hydatigena* was established in a medium and heavy experimental infestation in dog.

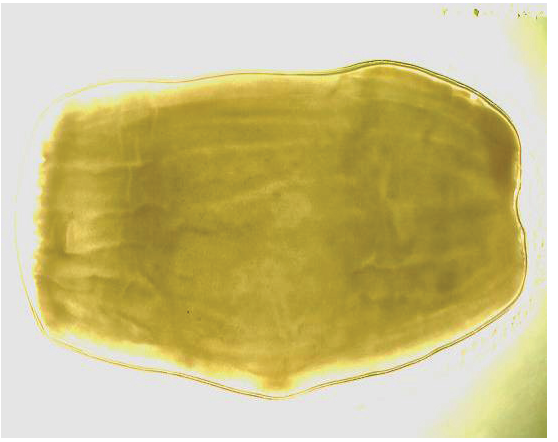

Fig. 2. Released gravid proglottid 8th week
magnification: 15X

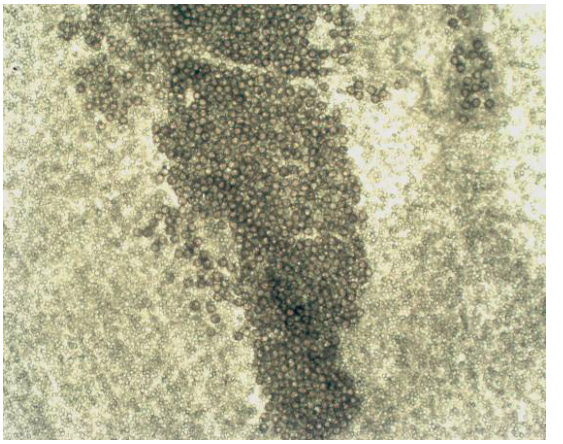

Fig. 4. Zone with taeniid eggs from released, gravid proglottid,
magnification: 40X

Fig. 3. Released gravid proglottid 12th week
magnification: 15X

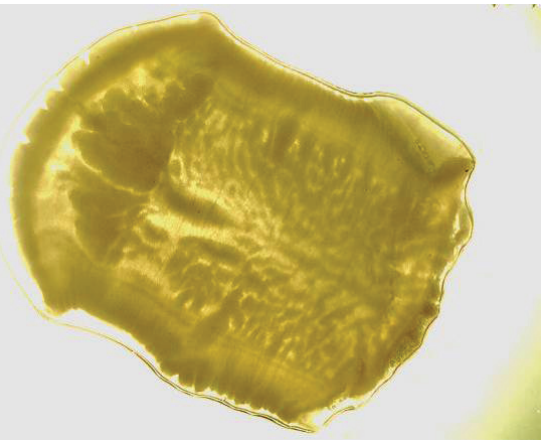

Fig. 5. Released gravid proglottid 13th
magnification: 15X

- It was confirmed for this taenia species that the gravid segments were highly movable and were spontaneously released from the anal opening of the final host.
- The average number of proglottids released daily and per specimen of *T. hydatigena* was established for the first 15 weeks of the patent period.
- The dynamics of the release of gravid *T. hydatigena* proglottids for the 15-week period was analyzed.
- The morphology of the uterus of the gravid segments was studied in details in the present research.

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