



## MORPHO-ANATOMICAL TRAITS OF *CYSTICERCUS PISIFORMIS* IN EUROPEAN RABBITS (*ORYCTOLAGUS CUNICULUS*)

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### Summary

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The study was conducted in the laboratory of the Department of Parasitology and Veterinary-Sanitary Examination of the Poltava State Agrarian University. In total, 154 slaughtered rabbits of the Grey Giant breed aged 3–4 months, reared at private farms of the Poltava region (Ukraine) were examined. The aim of the research was to establish differential morphological and metrical features of metacestodes isolated from domestic rabbits. Only one species, *Cysticercus pisiformis* was recorded; invasion rate was 17.53%. White-grey bubbles were found on the serous membranes of the abdominal and, less often, thoracic cavities. The ultrastructural characteristics of the main parts of the scolex, such as suckers, rostellum and hooks are given. The morphometric characteristics of metacestodes collected from male and female rabbits were slightly different. The study of chitinoid hooks had the greatest diagnostic value. A statistically significant variation was noted for large and small hooks. Thus, the average size of large and small hooks was likely to be smaller by 1.33 and 3.37%, respectively in samples obtained from female rabbits. The metacestodes isolated from female rabbits were larger than those obtained from male rabbits regarding length of the handle of the large (114.28 µm vs. 105.26 µm) and small hooks (43.06 µm vs. 39.3 µm, respectively).

**Key words:** cysticercosis, *Cysticercus pisiformis*, identification, morphometric parameters, rabbits

### INTRODUCTION

Currently the production of European rabbits (*Oryctolagus cuniculus*), mostly bred in private farms, is on the rise in Uk-

raine. The meat of these animals is a source of additional income for farmers. Breeding rabbits offers a stable supply of

useful meat products, valuable fur and technical raw materials. The successful development of this industry is due to the biological and economically useful features of rabbits: high fecundity, precociousness, easily met requirements of housing conditions (cages in the yard, sheds, barns), and involvement of different strata of population. The low price and effective use of seed, meadow and forest fodder with minimal expenditure of high-cost concentrated feed, premixes, etc. also facilitate the breeding of rabbits (Kotsubenko *et al.*, 2021).

Quite often veterinarians do not attend private farms and pay insufficient attention to parasitic infections, although they are common in animals. In addition to the widespread coccidiosis among rabbits (Ola-Fadunsin *et al.*, 2018; Prus & Duda, 2021), pisiform cysticercosis (*Cysticercus pisiformis*) is another helminthiasis that causes significant economic losses (Wang *et al.*, 2020; Arias-Hernández *et al.*, 2020). Thus, at birth, the size of the offspring of affected females was twice smaller compared to offspring of healthy females; accordingly, the weight of the rabbits at weaning was lower (Hallal-Calleros *et al.*, 2016). The authors claim that the effect was induced by increased levels of progesterone in the infested rabbits. At the same time, according to another study, the levels of glutamate dehydrogenase (EC 1.4.1.2) in the serum of rabbits infected with *Taenia pisiformis* eggs reflected the degree of liver parenchyma damage. In addition to its detoxification function, the liver takes part in the processes of digestion, haematopoiesis, the metabolism of vitamins, proteins, fats, and carbohydrates. As a result of impaired liver function, immunological reactivity in sick rabbits is compromised and they be-

come susceptible to re-infection with other pathogens (Rajasekaria *et al.*, 1985).

The cycle of development of cysticercosis has long been deciphered. Cestodiasis occurs as a result of ingestion of *T. pisiformis* eggs with food or water. Recent studies indicate the possibility of transmission of tapeworm eggs through arthropods, as they can remain viable for several months, allowing arthropods to mechanically transport them to intermediate hosts (Benelli *et al.*, 2021).

The circulation of the pathogen in wild fauna is thoroughly studied (Remesar *et al.* 2021; Samorek-Pieróg *et al.*, 2021). Thus, in Great Britain the invasion rate in foxes was 13.8% (Richards *et al.*, 1995). In Tunisia, Northern Africa the invasion rate of *Taenia pisiformis* in jackals was 3.2% (Lahmar *et al.*, 2014). Naturally, the intermediate host (the rabbit) is also infested with metacestodes. European rabbits are highly infested in Iraq, where the invasion rate was 68.18% (Daoud *et al.*, 2005). In Hagi, Yemen this parameter was even higher, 76.6% (Mogalli, 2020). In the southern regions of Ukraine rabbits are also highly infested with cysticercosis, and the rates increase proportionally to the age of the intermediate hosts. Thus, the extensity of invasion rates in rabbits aged 60 days was 23.62% compared to 67.14% in hosts aged 180 days (Bogach *et al.*, 2022). According to other data for the territory of Ukraine, cysticercosis infection rates in rabbits were 1.85% as monoinfection, and 19.64% as part of cysticercosis and eimeriosis co-infection (Prus & Duda, 2021).

Thus, taeniids is a very interesting, group of parasitic cestodes, unique in many ways. Data on the spread of the pathogen and its larval stages in domestic and wild animals are quite fully described in scientific works. Based on many years

of research, scientists suggested using both gene sequences and morphological features to identify parasites (Sun *et al.*, 2008; Chawhan *et al.*, 2016; Greben *et al.*, 2023). At the same time, many issues of the morphology and differential diagnosis of metacestodes proper to rabbits remain outdated.

Therefore, the aim of our research was to establish differential species-specific morphological and metric parameters of *Cysticercus pisiformis* obtained from European rabbits of the Gray Giant breed. In order to achieve the goal, it was necessary to establish the identifying morphological features of isolated cysticerci and to determine differential metric parameters and their dependence on the sex of the intermediate host.

#### MATERIALS AND METHODS

The research was conducted in the scientific laboratory of the Department of Parasitology and Veterinary-Sanitary Examination (Poltava State Agrarian University) in 2020–2022. All manipulations with experimental animals were carried out in compliance with ethical standards and in accordance with international and Ukrainian legislation. The protocol of the current study was approved by the Ethics Committee of the Poltava State Agrarian University (approval number: 2022/03). The rabbits were kept in private farms in the Poltava region, Ukraine. Experiments were conducted on 154 (93 females and 61 males) slaughtered Gray Giant rabbits aged 3–4 months. The metacestodes were collected by the method of complete helminthological dissection of individual organs (Zon, 2005). The larval stage type was established using the key of Ryzhikov *et al.* (1978). To study the ultrastructural characteristics of the main parts of the

scolex, metacestodes were placed in Hoyer's medium.

The specimens were examined and identified using Zeiss Axio Imager M1 light microscope in interactive mode using ×4 objective at I. I. Schmalhausen Institute of Zoology (Kyiv, Ukraine).

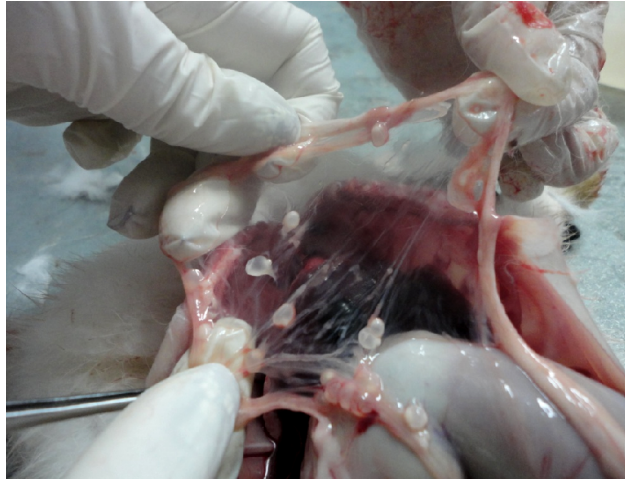
Statistical processing of the obtained research results was performed using the STATISTICA 10 software (StatSoft Inc., USA, 2011). Metacestode parameters were expressed as mean ± SD (standard deviation). The Shapiro–Wilk test was used to check the distribution of indicators for normality. *Post hoc* comparisons between groups were performed using the Mann-Whitney test. The dependence of invasion rate on the host sex was determined using the Chi-square test. Differences between indicators in groups were considered significant at  $P < 0.05$ .

#### RESULTS

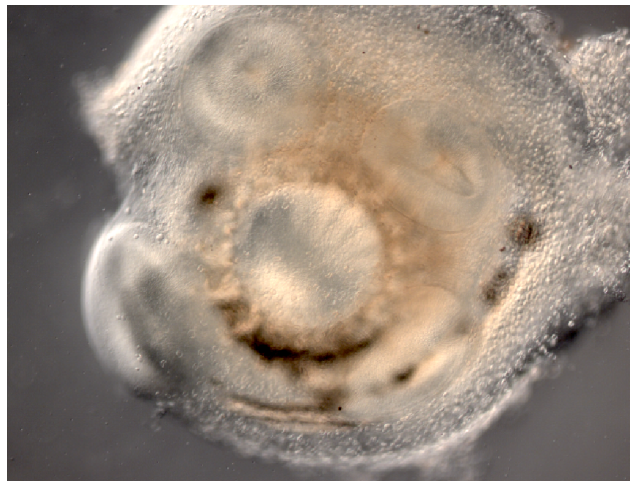
Only one metacestode species was recorded on private farms in the Poltava region (Ukraine). The total prevalence was 17.53%. Invasion rate was in the range of 14–89 larval cysts per animal. The conducted studies established that the frequency of damage to females by larval cysts was higher (19/93; EI 20.43%), compared to that seen in males (8/61; EI 13.11%).

During dissection of rabbits, white-grey bubbles were found on the serous lining of the abdominal and, rarely, thoracic cavities (Fig. 1). According to the results of parasitological studies, metacestodes isolated from rabbits were identified as *Cysticercus pisiformis* (Fig. 2).

The main metric indicators of metacestodes from rabbits of the Gray Giant breed were determined to update data on morphological features and differential



**Fig. 1.** The omentum of a rabbit invaded by cysticerci.



**Fig. 2.** General view of the scolex.

diagnosis (Tables 1 and 2). The oval-shaped cysticercus was 6–12 mm long and 4–6 mm wide. The wall of the cysticercus had two shells, the outer made of connective tissue and the inner of embryonic tissue. Inside, the cysticercus was filled with a clear fluid. The scolex, in the form of a white spot, showed through the wall. The width of the cysticerci obtained from male rabbits was by 6.59% smaller ( $P<0.05$ ) than the width of cisticerci col-

lected from female hosts. The cysticercus was surrounded by a capsule formed by the intermediate host tissue.

The main measurements of metacestodes depended on the sex of the animal host. The scolex length and the rostellum diameter were smaller by 13.29% ( $P<0.05$ ) and 13.52% ( $P<0.001$ ), respectively, in the samples collected from male rabbits.

The species identification of metacestodes was carried out by a number of systematic features including the morphometry of chitinous formations of the attachment apparatus. Double rows of rostellary hooks on the scolex were observed. The size of hooks and hook rows were different. The number of hooks varied significantly from 32 to 42. The hooks

were characterised by a specific morphological structure: the hooks of the first row were larger than those of the second row (Table 2). The latter had curved sharp blades. There were significant differences in the morphometric parameters of the hooks of both rows in cisticerci obtained from male and female hosts. Thus, the average lengths of large and small hooks

**Table 1.** Metric features of metacestodes obtained from male and female hosts. Data are presented as mean ± SD (n=10) and min–max range.

Features	Female host (n=10)			Male host (n=10)		
	mean ±SD	min	max	mean ±SD	min	max
Cysticercus length, µm	7305.0±743.3	6000.0	8500.0	7140.0±704.3	6000.0	8500.0
Cysticercus width, µm	4345.0±407.2	4000.0	5000.0	4060.0±343.8*	3700.0	5000.0
Scolex length, µm	3910.0±839.9	3000.0	6000.0	3390.0±931.5*	3000.0	6000.0
Scolex width, µm	1605.0±272.3	1300.0	2000.0	1775.0±272.1	1300.0	2000.0
Sucker length, µm	324.57±41.19	261.26	392.00	327.80±37.57	290.00	390.00
Sucker width, µm	209.26±60.37	126.27	316.23	239.50±44.75	190.00	310.00
Rostellum diameter, µm	552.72±38.96	486.00	597.00	478.0±44.17***	420.00	520.00

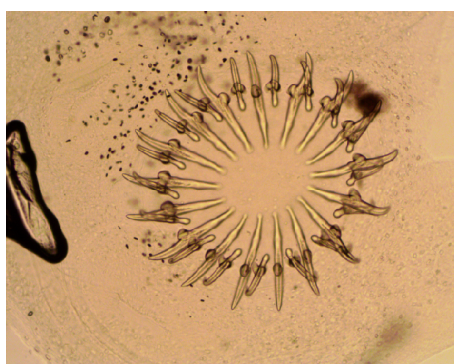
\* P<0.05; \*\* P<0.01; \*\*\* P<0.001 between groups.

**Table 2.** Metric features of hooks of cisticerci obtained from male and female hosts. Data are presented as mean ± SD (n=30) and min–max range.

Features	♀ (female host group)			♂ (male host group)		
	mean±SD	min	max	mean±SD	min	max
Large hook, µm:	216.04±10.47	191.21	233.05	218.95±23.09*	118.0	240.0
handle, µm	114.28 ±7.50	99.65	126.86	105.26±11.29***	80.0	128.0
blade, µm	82.02±5.76	63.7	90.71	81.26±6.75	68.0	95.0
guard, µm	106.72±5.69	97.66	124.35	117.76±8.9*	100.0	130.0
base, µm	34.98±5.88	25.92	49.06	36.13±5.35*	28.0	50.0
Small hook, µm:	124.81±6.81	111.0	135.38	129.16±9.51*	110.0	145.0
handle, µm	43.06±5.66	29.25	58.79	39.30±6.21*	25.0	50.0
blade, µm	64.70±3.87	56.63	72.52	69.06±14.91	55.0	118.0
guard, µm	30.24±3.63	21.77	37.52	33.90±4.99**	25.0	45.0
base, µm	82.34±3.99	74.29	92.91	93.06±8.24***	80.0	110.0

\* P<0.05; \*\* P<0.01; \*\*\* P<0.001 between groups.

were smaller by 1.33% ( $P<0.05$ ) and 3.37% ( $P<0.05$ ), respectively in samples collected from female hosts. At the same time, the base and root process of metacestode hooks isolated from male rabbits were larger (Fig. 3). The morphometric parameters of ♀ experimental group had higher values of lengths of the handle of small (by 8.73%,  $P<0.05$ ) and large hooks (by 7.89%,  $P<0.001$ ).



**Fig. 3.** Alternate arrangement of hooks in cysticercus metacestode.

## DISCUSSION

Although the modern rabbit farming is conducted using the latest technologies, which allow obtaining maximum profits, parasitoses as form of antagonistic trophic relationships have a negative effect on the condition of animals and can cause their death (Mykhailiutenko *et al.*, 2019). According to numerous studies, the presence of metacestodes in the body entails a chronic disease associated with long-term presence of the pathogen in the body. At the same time, clinical signs are weakly expressed, and pathoanatomical alterations are capable of actually changing the functional part of the organ. A significant role in the pathogenesis of helminthiasis is attributed to their mechanical impact,

due to the fixation of parasites (by suckers, hooks) and traumatic injury of host tissues or organs, as well as associated with the migration of larvae in the body (Mir *et al.*, 2006; Duda *et al.*, 2019). Thus, *Cysticercus pisiformis* (Bloch, 1780) mainly inhabits the liver capsule, peritoneum, large omentum and mesentery, and sometimes other organs, such as the lungs (Chen *et al.*, 2021). In one study, 39.1% of metacestodes were located in the liver (Kang, 1987). Bogach *et al.* (2022) found the largest number of cysticerci in the mesentery and omentum. At low intensity, they settled only on the serous membrane of the rectum. In 320-day-old rabbits, along with live cysticerci, the presence of degenerative *C. pisiformis* was recorded due to the different lifespan of the parasites and repeated infection of the animals. The authors noted that 42% of cysticerci were dark brown, which indicated their non-viability. Our data on the localisation of cysticerci in both female and male Gray Giant rabbits aged 3–4 months were similar: no dependence between the damage of rabbits by larval cysts and sex of the host was found, although the rate of infestation in male rabbits was by 7.32% lower. In our opinion, this fact is caused by a small sample of experimental animals.

The results of morphological studies play an important role in practical taxonomy for the construction of a natural system of parasites (Chervy, 2002; Radwan *et al.*, 2022). According to data obtained in Korea, the measured cysts were 7.04 mm in length and 4.62 mm in width (Kang, 1987). In this study, the average length of the cysticercus ranged from 7140.0 to 7305.0  $\mu\text{m}$ , and the width from 4060.0 to 4345.0  $\mu\text{m}$ , depending on the sex of the rabbit. Ryzhikov *et al.* (1978) found 38 to 48 hooks on scolex. In our

study, their number was in the range from 32 to 42. In particular, 16 to 19 large hooks and 16 to 23 small ones were detected. In another study, 37 hooks in the cyst of *Cysticercus pisiformis* were mentioned (Sun *et al.*, 2008).

The researchers declared the need to take into account the morpho-anatomical features of hooks in order to differentiate metacestodes. The comparative size of long and short chitinous hooks have the greatest diagnostic value (Esch *et al.*, 1965). Subsequently, other researchers counted the total number of hooks (large and small) in *Cysticercus cellulosae* to differentiate *C. viscerotropica* (Chawhan *et al.*, 2016). Therefore, microscopic examination of metacestodes highlights a typical characteristic for identification. As a result of morphometry, scientists from Korea established the length of large (on average 250 µm) and small (on average 150 µm) hooks of *Cysticercus pisiformis* (Kang, 1987). In another study the cross-sectional diameter of the hook (0.79–1.32 µm) was determined (Sun *et al.*, 2008).

In this study, we have updated the outdated and extremely limited data of morphological parameters in the conditions of Poltava region, Ukraine. The data demonstrated a reliable difference in ultrastructural characteristics of hooks, such as the length, handle, blade, guard and base of hook, depending on the sex of rabbits. Such research was conducted for the first time in Ukraine. Differences in the structure of *Cysticercus pisiformis* characterise the morphological adaptation of the parasite depending on a number of factors: host sex, climate, ecology, geographical location.

Therefore, the present study of the distribution and morphometric characteristics of *Cysticercus pisiformis* in rabbits in the Poltava regions of Ukraine allows adding

new data on the variability of the metacestodes' size in rabbits. We also suggest taking into account the above-mentioned metric indicators of hooks in the identification of metacestodes in rabbits.

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