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Article · January 2009

DOI: 10.2478/v10058-009-0019-5

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Vestnik zoologii, **43**(5): 433–440, 2009
DOI 10.2478/v10058-009-0019-5

UDC 595.133:598.1+591.8

ON THE MIGRATION OF CYSTACANTHS *SPHAERIROSTRIS PICAE* (ACANTHOCEPHALA, CENTRORHYNCHIDAE) IN PARATENIC HOST *LACERTA AGILIS*, HISTOPATHOLOGY

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Accepted 7 August 2009

On the Migration of Cystacanths *Sphaerirostris picae* (Acanthocephala, Centrorhynchidae) in Paratenic Host *Lacerta agilis*, Histopathology. Krasnoshchekov G. P., Lisitsyna O. I. — The penetration mechanism of *Sphaerirostris picae* cystacanths in intestinal wall of the paratenic host *Lacerta agilis* and characteristic features of inflammatory changes in host intestinal wall are examined. Inflammatory reaction is characterized by predomination of eosinophiles and comparatively small amount of neutrophiles. The reaction is much more pronounced when cystacanth is placed in parallel to the wall of intestine. Extensive edema, plethora, stases in vessels, and slight hemorrhages are observed in submucous membrane. Inflammatory infiltration is less pronounced in case of perpendicular orientation of the proboscis. The secretory glands in cystacanths were not found, secretion on the tegumental surface was not seen, as well as no morphological effect of lytic enzymes on host tissues. The penetration of host intestinal wall is shown to be mechanic, advancement of the cystacanth through the wall of intestine is due to alterations of partial evagination and invagination of the presoma in combination with contractions of the metasoma.

Key words: acanthocephala, cystacants, paratenic, hosts, histopathology, migration.

К вопросу о миграции цистакантов *Sphaerirostris picae* (Acanthocephala, Centrorhynchidae) в паратеническом хозяине *Lacerta agilis*, гистопатологический аспект. Краснощеков Г. П., Лисицына О. И. — Авторами изучен механизм пенетрации цистакантами *Sphaerirostris picae* кишечной стенки паратенического хозяина *Lacerta agilis* и особенности воспалительных изменений стенки кишечника хозяина. Воспалительная реакция характеризуется преобладанием эозинофилов при относительно небольшом числе нейтрофилов и наиболее выражена при параллельной ориентации цистаканта относительно кишечной стенки. При этом в подслизистой отмечается распространенный отек, полнокровие и стазы в сосудах, небольшие кровоизлияния, диффузная воспалительная инфильтрация. Наименее воспалительная инфильтрация выражена при перпендикулярной ориентации хоботка. Секреторные железы у цистакантов не обнаружены, признаки выделения секрета на поверхность тегумента не отмечены, как и морфологические проявления воздействия литических энзимов на ткани хозяина. Показано, что пенетрация кишечной стенки хозяина осуществляется механически, продвижение цистаканта через стенку кишечника обеспечивается чередованием частичной эвагинации и инвагинации пресомы в сочетании с сокращениями метасомы.

Ключевые слова: акантоцефалы, цистакант, паратенический, хозяин, гистопатология, миграция.

Paratenic parasitism is determined by ability of invasion stages of zooparasites from different systematic groups to penetrate the digestive tract wall of unusual hosts and to settle in different organs and tissues (Sharpilo, 1971 and others). The mechanism of migration is peculiar for every group of parasites and depends on the presence of special morphological formations (spikes, hooks, etc.) and penetrative glands. Similar modified morphological structures are present in plerocercoids of some cestodes (Kuperman, 1980 and others), miracidia, cercaria and mesocercaria of many trematodes (Ginetsinskaya, 1968 and others), some nematodes, e. g., in larvae of *Gnathostoma* spp. (Anderson, 2000).

Infective larvae of many acanthocephalans (cystacanths) are capable to paratenic parasitism, however, the mechanism of cystacanth migration in paratenic hosts is unknown. Numerous studies of presoma thin structure in different types of acanthocephalans (Miller, Dunagan, 1985) did not reveal any expressed glandular morphological structures. In embryonic larvae of acanthocephalans, glandular granules were found in anterior part of the body, and acanthors use their secretion during penetration of the digestive tract of intermediate hosts (Nikishin, Krasnoschekov, 1990; Nikishin, 2004). Though acanthella have no glandular granules yet, it is possible to suggest something similar in cystacanths. On the other hand, anchoring organ in cystacanths and adult acanthocephalans is usually equipped with great number of powerful hooks suggesting mechanical penetration of intestinal wall in paratenic hosts.

The purpose of this work is to reveal the mechanism of cystacanthic penetration of intestinal wall in paratenic hosts.

Material and methods

The series of experiments on infection with cystacanths *Sphaerirostris picae* (Rudolphi, 1819) Golvan, 1956 (Acanthocephala, Centrorhynchidae) of sand lizards *Lacerta agilis* Linnaeus, 1758, usual paratenic hosts of this species of acanthocephalans, were carried out. Cystacanths (12 specimens, first series of preparations) were fixed on successive stages of penetration of lizards' intestine wall. Fragments of intestines with cystacanths anchored in it were fixed in Buen's liquid, dehydrated and mounted in paraffin; microscopic sections of 5—7 microns thick were made. Also, for comparison, the sections were made from cystacanths of *S. picae* extracted from the body of sand lizards caught in nature (cystacanths were released from envelopes and rostella were evaginated; 6 specimens, second series of preparations). Sections were stained with Gomori hematoxylin-eosin and alcian blue.

Results and discussion

The top of proboscis in all cystacanths from the first experimental series was more or less invaginated. Such state of proboscis can not be explained by contraction of proboscis muscles during fixation only. The initial stage of rostellum invagination after penetration of abdominal cavity should also be excluded because none of cystacanths perforated intestinal wall. We suggest that cystacanth proboscis did not evaginated completely during penetration. Also, in adult acanthocephalans anchored to intestinal mucosa of definitive host, proboscis is usually more or less invaginated (Taraschewski, 2000).

Cystacanths are submerged into the host intestinal wall and oriented in parallel, obliquely or athwart to it. The greater part of cystacanth oriented in parallel is in submucous membrane between mucous and muscular layer; cystacanth, oriented obliquely, gets into the muscular layer to various depth; being oriented athwart, it penetrates all depth of host intestinal wall except for serous membrane. It is obvious that cystacanths oriented athwart or close to it finish penetration quicker than those oriented obliquely, and cystacanths parallel to the host intestinal wall may not finish migration and persist in connective tissue between muscular and subserous layers of host intestinal wall. Such localization of cystacanths is rather often mentioned in literature (Sharpilo, 1976) and we often observed it during dissection of reptiles. On the average, actual penetration of the host intestinal wall takes up to 8—10 hours; period from feeding of cystacanths until they got into abdominal cavity is no less than 2 days.

Histopathology of abdominal organs in fish paratenic hosts caused by cystacanths was described by O. Amin et al. (1995, 1996). Inflammatory changes around cystacanths penetrating intestine wall of lizards have some peculiarities. Their variations can be explained by different rate of penetration according to cystacanths orientation relatively to host intestinal wall. Minimum inflammatory changes in intestinal wall are seen when proboscis is directed athwart (fig. 1). In such cases, slight edema is noted in submucosa and connective tissue between muscular and subserous layers of intestinal wall immediately near the proboscis. Inflammatory infiltration is developed poorly, especially in muscular layer and loose connective tissue layer under serous membrane. Mesothelial cells are flattened, of usual appearance. When cystacanth is directed obliquely, edema, dilated lymphatic vessels, perivasculitis and focal eosinophile accumulations are seen in submucosa (fig. 2, *b*). Increased mucus content is revealed on epithelial surface; mucin containing cells in areas of mucous membrane adjacent to the

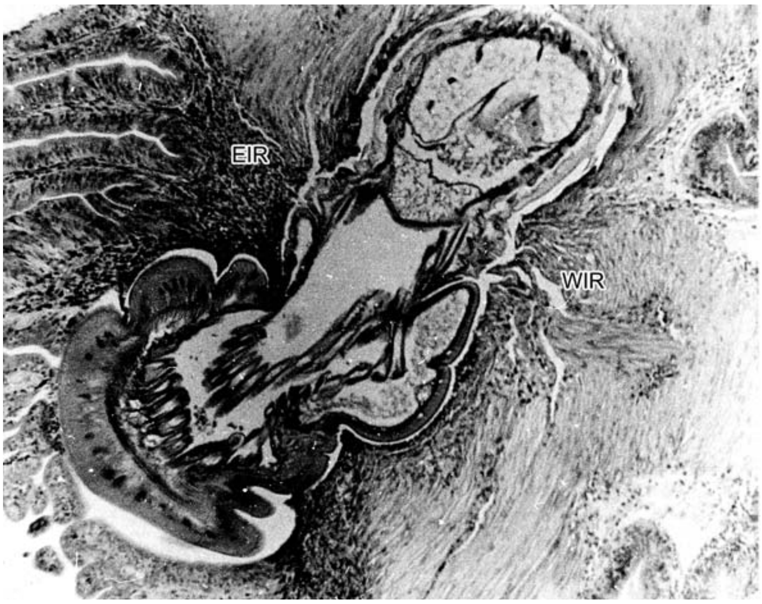

Fig. 1. Almost perpendicular orientation of cystacanth in relation to intestinal wall. Expressed inflammatory reaction (EIR) in submucous membrane; weak inflammatory reaction (WIR) in muscular layer. Metasoma compressed, lacunary system poorly seen. Magn. 10x7.

Рис. 1. Близкая к перпендикулярной ориентация цистаканта относительно стенки кишечника. Выраженная воспалительная реакция в подслизистой (EIR); слабая в мышечном слое (WIR). Метасома сжата, система лакун просматривается слабо. Ув. 10x7.

larva are not numerous or can not be revealed by staining for acid mucopolysaccharides. Inflammatory reaction is much more pronounced when cystacanth is placed in parallel to the wall of intestine. Extensive edema, plethora, stases in vessels, and slight hemorrhages are observed in submucous membrane. Also, diffuse inflammatory infiltration is often emerged on considerable distance from cystacanth and, occasionally, proliferation of loose connective tissue is observed (fig. 2, *a*). Eosinophils prevailed among infiltration cells, with especially great number in the place of extensive inflammatory process. Many eosinophils are on different stages of degranulation. Infiltration contains relatively small amounts of macrophages, cells similar to plasmatic and lymphoid cells, neutrophils. When proboscis penetrates serous membrane, there are similar changes in it, but cellular infiltration is expressed much weaker with eosinophils predominated. Mesothelial cells above the proboscis are turgid, ovoid or pear-shaped, sometimes placed in few rows, closely to each other and forming continuous layers. When proboscis penetrates the abdominal cavity, sometimes granulation tissue excrescence is seen above it with expressed inflammatory infiltration, but frequently the proboscis surface turned into abdominal cavity is clean with no adhesion of host cells on it.

Consequently, inflammatory changes around penetrated cystacanths have some characteristic features. Their different intensity probably depends on parasite orientation in relation to the host intestinal wall, time of contact of parasite with host tissues and determined by protective mechanisms of each layer of host intestinal wall reacting on parasite invasion. Distinctive feature of inflammatory reaction, even in the most expressed cases, is prevalence of eosinophils and relatively low number of neutrophils. Taking into account mechanical injuries of host tissues made by proboscis hooks of parasite and concomitant microbiosis, we might expect that inflammatory reaction when cystacanths penetrate intestine would be purulent, but not productive inflammation as it is. The course of inflammatory reaction is explained by secretory activity of parasite's tegument probably realized from the membranocalyx surface or through the pores of

Fig. 2. Inflammatory reaction in different cystacanth orientation in relation to the host intestinal wall. *a* — orientation of cystacanth in parallel to intestinal mucous membrane. Intensive inflammatory infiltration (III) with predominating eosinophils in submucous membrane. Dilated lymphatic vessels in villi (HVV), perivasculitis; *b* — rostellar penetration of intestinal wall at slanting cystacanth orientation. Expressed inflammatory reaction in submucous (EIRM) and subserous (EIRS) membranes. Proliferative inflammation (PI) on the surface of serous membrane around the rostellum. Expressed lacunas (L) in coverings of metasoma, anterior part of soma. Magn. 16x7.

Рис. 2. Воспалительная реакция при различной ориентации цистаканта относительно слизистой кишечника хозяина. *а* — ориентация цистаканта параллельно слизистой кишечника. Интенсивная воспалительная инфильтрация (III) с преобладанием эозинофилов в подслизистой. Расширение лимфатических сосудов ворсинок (HVV), периваскулит; *b* — пенетрация хоботком стенки кишечника при косой ориентации цистаканта. Выраженная воспалительная реакция в подслизистой (EIRM), субсерозной (EIRS) оболочках. Пролиферативное воспаление на поверхности серозной оболочки вокруг хоботка (PI). Выраженные лакуны (L) в покровах метасомы, передних отделах сомы. Ув. 16x7.

channels in the outer glycocalyx layer. There are evidences in literature on modifying effect of parasite, in particular, on production of substances inducing eosinophilic taxis and, obviously, inhibiting neutrophilic migration (Linghtowlers, Rickard, 1988; Taraschewski, 2000). However, additional researches with using of electron microscopy and histochemical techniques are needed to specify and confirm such a phenomenon.

Structure and composition of acanthocephalic integumentary tissues, including those on cystacanth stage, are described in detail (Miller, Dunagan, 1985; Nikishin, 2004). We draw attention to characteristics that are likely connected with cystacanth's penetration of host intestinal wall.

Lacunary system is different in different individuals. In cystacanths with compact metasoma and almost completely evaginated proboscis, lacunas are not viewed (fig. 1). In internal parts of tegument, near basal membrane, cytoplasm is fragmented forming

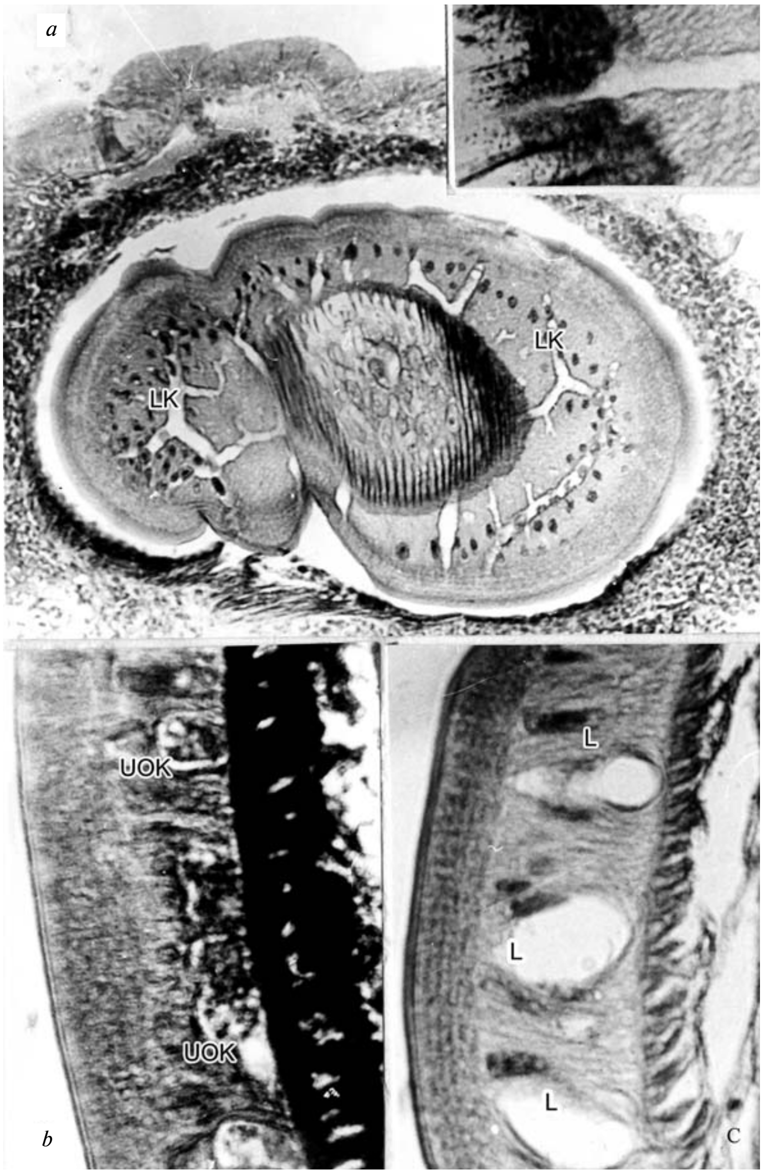

Fig. 3. Different state of lacunas of cystacanths: *a* — tangential section; system of lacunas and channels (LK) in the posterior part of soma (magn. 10x7); inset — continuation of channel into the larva's body; *b* — Fragmentation of cytoplasm with formation of unclearly outlined cavities (UOC) filled with grainy mass; *c* — large multiple lacunas (L) in cystacanth's tegument from the host abdominal cavity (magn. 40x7).

Рис. 3. Различное состояние лакун цистакантов: *a* — тангенциальный срез; система лакун и каналов (LK) в заднем отделе сомы (ув. 10x7); врезка — продолжение канала в полость тела личинки; *b* — фрагментация цитоплазмы с образованием нечетко контурированных полостей (UOC), заполненных зернистой массой; *c* — крупные множественные лакуны (L) в тегументе цистаканта из брюшной полости хозяина (ув. 40x7).

unclearly outlined cavities filled with grainy mass (fig. 3, *b*). In other areas, lacunas are seen more distinctly, they cross parts of tegument and end in middle ones, not getting into them significantly. In cystacanths with almost spread metasoma and considerably invaginated proboscis, lacunas are more numerous, in the form of cavities (fig. 2, *b*) or, on tangential sections, they look like network of longitudinal and radial channels lacking the content, as a rule (fig. 3, *a*). Sometimes, these channels are seen to get through the basal membrane and their lumen communicates with cystacanth's cavity (fig. 3, insert). Most expressly lacunary system is seen in cystacanths extracted from abdominal cavity of naturally infected paratenic hosts (preparations from series 2) and fixed with completely evaginated rostellum and spread metasoma (fig. 3, *c*). Proboscis tegument is thinner, its internal layer is empty, external layer is fine-grained, layer's thickness is various (fig. 4).

We should note that cystacanth as invasion larva is fully formed in the body of intermediate host and is juvenile individual with all organs and tissues developed (Petrochenko, 1956; Amin et al., 1995). In this connection, we consider that different state of lacunas on different stages of penetration are rather the evidence of its active functioning than display of formed lacunary system.

Signs of secretion on tegumental surface were not noted, secretory glands in cystacanths were not found and morphological effect of lityc enzymes on host tissues was not seen. It suggests that cystacanths penetrate the host intestinal wall mechanically. During penetration, both presoma with its hooks and musculature and metasoma with extended lacunar system and strong muscular system are likely participating.

This process can be described sequentially as follows: in the digestive tract of paratenic host, the envelope surrounding cystacanth is broken, cystacanth partly evaginates its proboscis and anchors into intestinal mucosa with the aid of hooks on its subapical or middle part. Metasoma is slacked, lacunas are filled with liquid. Then subapical part of proboscis invaginates further while the adhered and adjoining areas of intestinal mucous membrane tightly surround middle part of proboscis and wrenched hooks of this part of proboscis. Next stage — due to efforts of presomal and metasomal muscles, contraction of lacunas and moving of liquid contained in them from the distal parts of the body to parasite's presoma, proboscis is turned inside out tearing host

Fig. 4. Hooks tear host tissues. Rostellar tegument (RT). Rarefaction of matrix in external parts, emptiness in internal part. Eosinophilic infiltration in adjacent host tissues. Magn. 40x7.

Рис. 4. Крючья разрывают ткани хозяина. Тегумент хоботка (RT). Разрежение матрикса наружных отделов, опустошение — внутренних. Инфильтрация эозинофилами прилежащей ткани хозяина. Ув. 40x7.

tissues by subapical and approximate hooks (fig. 4), and anchoring by the middle ones; metasoma compresses, "pulling up" to presoma and "pushing" it through into the walls of host intestine (fig. 1). Altered partial evagination and invagination of presoma in combination with contractions of metasoma provide advancement of cystacanth through the host intestinal wall. At the moment when opening appears in intestinal wall, cystacanth gets into abdominal cavity. Here it invaginates the proboscis and the capsule is formed around the cystacanth, probably made by host fibroblasts (the capsule structure is studied in *Corynosoma* sp. cystacanths from fishes) (Nikishin, Skorobrekhova, 2007). In such state the cystacanth is able to persist in paratenic host for rather long time. Interestingly, that the cycles of motions similar to those described here for cystacanths were observed in embryonic larvae (acanthors) of acanthocephalans in the intestine of intermediate host (Taraschewski, 2000).

Offered version of penetration mechanism of cystacanths *Sphareirostris picae* into intestinal wall in digestive tract of sand lizard is experimentally supported illustration of the process taking place in the body of host cold-blooded animal.

Warm-blooded animals, in particular insectivores, can serve as paratenic hosts for many acanthocephalans including representatives of the family Centrorhynchidae (Tkach, 1989; Kirillova, Kirillov, 2007). Penetration of intestinal wall of warm-blooded animal hosts occurs in similar way, but for the shorter period of time. Our small experiment can be the partial confirmation for it. Cystacanths of *S. picae* (2 specimens) were experimentally fed to a goldish hamster (*Ochrotomys nuttalli*) and in two days they were found in the body cavity of experimental hosts, with invaginated proboscis and being surrounded with thick capsules. To further, more detailed study of penetration into a warm-blooded animal, reaction of such a host, process of capsule formation around a parasite, additional researches are needed with using of electron microscopy and histochemical techniques.

Authors acknowledged Victor Sharpilo for the idea of such an experiment, Vladimir Nikishin for support, Vadim Kornyushin for participation and remarks to the manuscript.

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