强壮粗体虫寄生引起的鳜肠道病理

谢海侠 高 谦 聂 品
(中国科学院水生生物研究所; 淡水生态与生物技术国家重点实验室, 武汉 430072)

摘要: 强壮粗体虫是鳜肠道最常见寄生蠕虫。通过光镜及电镜对自然感染强壮粗体虫的鳜肠道进行了组织病理观察。强壮粗体虫的寄生引起鳜肠上皮细胞脱落、肠固有膜层结缔组织增生及白细胞向病灶处浸润, 并可观察到嗜酸性粒细胞附着在与肠上皮及固有膜接触的虫体的体壁及吻部。在虫体吻部与肠固有膜层接触处依次观察到纤维细胞、成纤维细胞、嗜酸性粒细胞等, 其中嗜酸性粒细胞又可分为未成熟嗜酸性粒细胞、成熟的嗜酸性粒细胞及正在脱颗粒的嗜酸性粒细胞。另外在肠壁的固有膜层观察到包裹虫体的结缔组织纤维囊, 囊壁由三层结构构成, 同时在鳜肠壁相同的位置观察到被宿主细胞浸润了的组织空腔, 推测其为结缔组织纤维囊退化所形成。

关键词: 棘头虫; 强壮粗体虫; 鳜; 肠道病理
中图分类号: S941.52 文献标识码: A 文章编号: 1000-3207(2005)02-0137-05

鳜[*Siniperca chuatsi* (Basilewsky)]是重要的名优淡水养殖鱼类, 其产量逐年上升, 然而由于疾病所导致的经济损失日益增长, 因此鳜鱼病害的病原生物学及防治研究倍受关注[1,2,3]。隶属新棘吻科(Neoechinorhynchidae)的强壮粗体虫(*Hebesoma violentum* Van Cleave, 1928)成虫寄生于鳜肠道, 是鳜肠道最常见的寄生蠕虫[4]。作者于2000年1月和2001年1月在湖北省牛山湖、小[illegible]湖、红莲湖和梁子湖对鳜肠道寄生蠕虫进行了调查, 发现强壮粗体虫的感染率均接近100%, 感染强度最高达112条/尾。参考鳜肠组织学的研究结果[5], 虫体主要分布于靠近幽门盲囊的小肠前段。目前关于鱼类棘头虫的报道仅限于其生态学研究[6,7], 尚未见到有关棘头虫寄生引起宿主组织病理方面的报道[8,9]。因此本文从光镜及电镜水平对强壮粗体虫寄生引起的鳜肠道病理进行了研究。

1 材料和方法

实验所用20尾鳜, 尾叉长20—25cm, 取自湖北梁子湖, 活体运回实验室。解剖鱼体后, 取出肠道并纵向剖开, 在解剖镜下观察后, 参照Reite的方法[10], 将鳜肠道固定于MFAA(甲醇∶福尔马林∶乙酸=85∶10∶5)(18—24h, 4℃)。常规石蜡包埋, 对整个包埋块进行连续切片, 二甲苯脱蜡后, 复水到70%乙醇, 麦格氏吉姆萨(May-Grünwald Giemsa)染色3h, 然后分别用蒸馏水(2sec)与无水乙醇(3sec, 2次)分色, 不经二甲苯透明, 直接用加拿大树胶封片, 在光学显微镜(Olympus BH-2)下观察并拍照。

另取自然感染强壮粗体虫的鳜肠道, 用生理盐水(4℃)洗净肠道内容物后, 平铺于滤纸上, 修剪成2mm×1mm×1mm^{3}的小块, 连同滤纸用2.5%的戊二醛固定(4℃, 12h), 用0.15mol/L, pH7.2磷酸缓冲液配制的1%的锇酸作后固定(4℃, 1.5h), 梯度酒精和丙酮脱水, Spurr树脂包埋。半薄切片(0.5μm)贴片于载玻片上, 1%的甲苯胺蓝染色, 于光学显微镜下定位。超薄切片(80nm), 经常规处理后, 在透射电子显微镜(JEOL JEM-1230型)下观察并拍照。

2 结果

组织切片观察发现, 在强壮粗体虫寄生部位, 鳜肠壁上皮细胞层变薄或消失, 并在虫体吻部跟肠壁固有膜层相接触处观察到白细胞浸润(图版Ⅰ: 1)。虫体吻部伸入肠壁固有膜层, 从而引起固有膜层结缔组织增生(图版Ⅰ: 2)。透射电镜观察发现在跟虫体吻部相接触的固有膜层中, 由近及远依次分布着纤维细胞、成纤维细胞以及淋巴细胞、嗜酸性粒细胞

收稿日期: 2003-10-20; 修订日期: 2004-05-18
基金项目: 国家杰出青年科学基金(30025035)资助
作者简介: 谢海侠(1976—), 女, 陕西宝鸡人; 博士研究生; 主要从事鱼类免疫学研究
通讯作者: 聂品(1961—), 男, 研究员; 主要从事鱼类寄生虫学及鱼类免疫学研究。Tel: 027-68780736, E-mail: pinnie@ihb.ac.cn

和巨噬细胞(图版Ⅰ:3)。

在虫体寄生部位的鳜肠壁上皮层和固有膜层中,可观察到深染嗜酸性粒细胞的聚集,一些嗜酸性粒细胞附着在与宿主肠壁相接触的虫体体壁上(图版Ⅰ:4)。在未观察到虫体的组织凹痕附近也可观察到肠上皮层中粘液细胞增生和固有膜层嗜酸性粒细胞、巨噬细胞和淋巴细胞的聚集(图版Ⅰ:5)。超微结构观察显示,寄生部位的嗜酸性粒细胞可大致分为三类,即未成熟的嗜酸性粒细胞,细胞核位于胞质边缘,胞质中可见电子致密颗粒、中等电子致密颗粒和电子稀疏颗粒,并分布有线粒体和发达的微管系统(图版Ⅰ:6);成熟的嗜酸性粒细胞,细胞核位于胞质边缘,核质比小,胞质中可见大量电子致密颗粒分布,但未见线粒体等细胞器(图版Ⅰ:7);脱颗粒的嗜酸性粒细胞,细胞核位于细胞中部,核质比较大,可见分布在胞质边缘以及存在于胞外的电子致密颗粒(图版Ⅰ:8)。

在鳜肠壁的固有膜层中,观察到两种形态的结缔组织纤维囊,一种囊仅由环绕强壮粗体虫的成纤维细胞构成(图版Ⅰ:9);另一种囊具有三层结构:由成纤维细胞环绕分布构成囊的外层,包绕虫体的纤维状物质构成囊的内层,内、外层之间的组织空腔为无定形物质填充(图版Ⅰ:10)。另外,还在肠壁固有膜层观察到一些不规则的囊状空腔(图版Ⅰ:11)。

3 讨论

宿主受到病原侵袭后,会通过产生炎症反应来清除病原[11]。宿主感染寄生虫后,机体内通常会产生趋化因子来吸引嗜酸性粒细胞迁移至寄生虫感染部位,因此宿主组织中嗜酸性粒细胞的迁移、聚集和增生一般被看作是蠕虫感染的特征性炎症反应[12]。另一方面,寄生虫成虫的寄生往往能导致宿主产生特异性的血清抗体[13],抗体和补体结合后,能通过补体受体与嗜酸性粒细胞相结合,跟抗体结合的补体介导嗜酸性粒细胞的脱颗粒[14],在嗜酸性粒细胞脱颗粒过程中释放的过氧化物酶等活性物质对寄生虫产生毒性杀伤作用,并可能是寄生虫从寄生部位脱落或迁移的原因[14,15]。嗜酸性粒细胞(Eosinophilic granular cells, EGCs)参与了棘头虫(*Acabtgocephalus jacksoni*)感染所导致的虹鳟(*Oncorhynchus mykiss*)肠道炎症反应。本研究结果表明,嗜酸性粒细胞也参与了强壮粗体虫感染所导致的鳜肠道炎症反应。但尚不清楚感染强壮粗体虫的鳜鱼是否产生了导致特异性的体液免疫反应,以及是否有抗体-补体复合物介导强壮粗体虫表面附着的嗜酸性粒细胞脱颗粒。另外,附近有大量白细胞聚集的组织凹痕可能是炎症反应致使强壮粗体虫发生迁移或脱落所留下的。

由于寄生虫个体较大,宿主免疫系统的吞噬细胞难以直接吞噬,而且许多寄生虫都发展了针对宿主免疫反应的逃避机制,因此得以继续存活,但在组织中存活的寄生虫往往最终被炎症反应产物所囊化,囊化可作为物理屏障,屏蔽宿主的炎症细胞对寄生虫的攻袭,同时也使宿主免受虫体分泌或排泄产物的毒害,寄生虫和宿主也因此而不再相互损伤[11]。圆桶新棘吻虫(*Neoechinorhynchus cylindratus*)的囊壁由胶原纤维构成内层,成纤维母细胞构成囊外层,从形态上看,强壮粗体虫囊壁的结构与圆桶新棘吻虫的结构是一致的。鲤新棘吻虫可以在似鲤形亚口鱼(*Carpiodes cyprinus* Lesueur)的肠壁形成结缔组织囊,且从肠上皮层到浆膜层,囊的血管化及组织增生程度增高[8]。本研究仅在鳜肠壁的固有膜层中发现包绕强壮粗体虫的结缔组织囊,且无论囊的结构是简单还是复杂,都未见血管分布其中。这些结缔组织囊的结构差异可能反映了其囊化程度的差别,具有明显三层结构的囊可能是其中囊化程度较高的类型。Crompton 认为囊化是一种慢性炎症反应,而退化是其可能的后续过程,故推测在固有膜层所观察到的囊状组织空腔为处在慢性炎症反应后期的结缔组织纤维囊形态。

参考文献:

[1] Gao Q, Nie P, Yao W J. Evidence of host blood feeding by the monogenean, *Ancyrocephalus mogumdae* (Monogenea: Ancyrocephalidae) from the gills of the mandarin fish, *Siniperca chuatsi*[J]. *Folia Parasitol*, 1999, **46**: 107—110

[2] Sun B J, Nie P. The outer membrane protein and lipopolysaccharide of *Cytophaga columnaris* and their immunogenicity to mandarin fish *Siniperca chuatsi*[J]. *Acta Hydrobiol Sin*, 2001, **25**: 524—527[孙宝剑, 聂品. 柱状嗜纤维菌的外膜蛋白和脂多糖及其对鳜鱼的免疫源性. 水生生物学报, 2001, **25**: 524—527]

[3] Wu S Q, Li X H, Pan H J, *et al*. Research on the pathogen of the outbreak-infective disease of *Siniperca chuatsi*[J]. *J Fish China*, 1997, **21**: 56—60[吴淑勤, 李新辉, 潘厚军, 等. 鳜爆发性传染病病原研究. 水产学报, 1997, **21**: 56—60]

[4] Pan J H, Zhang J Y, Li Z C. Fish Parasitology[M]. Beijing: Science Press, 1990[潘炯华, 张剑英, 黎振昌. 鱼类寄生虫学. 北京: 科学出版社, 1990]

[5] Yang X P, Zou H, Zhao A P. A preliminary study on the histology of digestive tract for the *Siniperca chuatsi* (Basilewsky)[J]. *J Huazhong Agr Univ*, 1993, **12**: 58—63[杨秀平, 邹海, 赵爱平. 鳜鱼消化

道组织学的初步研究. 华中农业大学学报, 1993, **12**: 58—63]

[6] Yang T B. Studies on the site selection of Acanthocephalan worms of fishes in the intestine of hosts in the Qinghai Lake[J]. *Acta Scientiarum Naturalium Universitatis Sunyatseni*, 1995, **34**: 79—83[杨廷宝. 青海湖鱼类寄生棘头虫在宿主肠中的位置选择. 中山大学学报, 1995, **34**: 79—83]

[7] Zeng B P, Liu X H, Tan L J, *et al*. A study on the infection of the finless eel (*Monopterus albus*) along the Xiangjiang River by acanthocephalan *Pallisentis* (*Neosentis*) *celatus* [J]. *J Xiangtan Normal Univ*, 2000, **21**: 91—96[曾伯平, 刘星辉, 谭立军, 等. 湘江水系黄鳝体内新棘衣棘头虫的感染研究. 湘潭师范学院学报, 2000, **21**: 91—96]

[8] Alexander J S, Terry A D. Intestinal pathology and site specificity of the acanthocephalan *Neoechinorhynchus carpiodi* Dechtiar, 1968, in Quillback, *Carpiodes cyprinus* (Lesueur)[J]. *J Parasitol*, 1987, **73**: 467—475

[9] McDonough J M, Gleason L N. Histopathology in the rainbow narter, *Etheostoma caeruleum*, resulting from infections with the acanthocephalans, *Pomphorhynchus bulbocolli* and *Acanthocephalus dirus*[J]. *J Parasitol*, 1981, **67**: 403—409

[10] Reite O B. Mast cell/Eosinophilic granule cells of teleostean fish: a review focusing on staining property and functional responses[J]. *Fish Shellfish Immunol*, 1998, **8**: 489—513

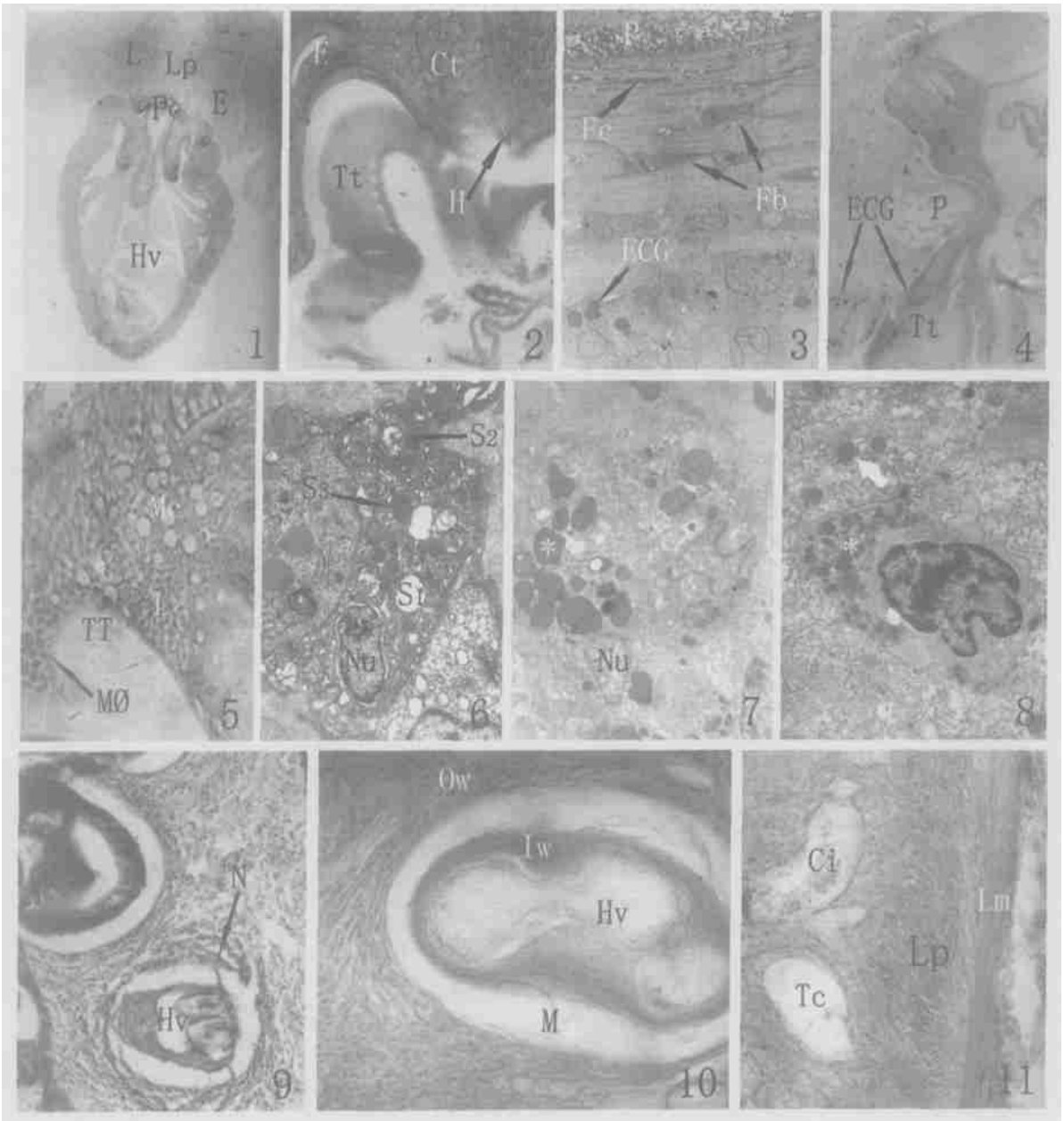
[11] Sharp G J E, Pike A W, Secombes C J. Sequential development of the immune response in rainbow trout [*Oncorhynchus mykiss* (Walbaum, 1792)] to experimental plerocercoid infections of *Diphyllobothrium dendriticum* (Nitzsch, 1824)[J]. *Parasitology*, 1992, **104**: 169—178

[12] Secombes C J, Chappell L H. Fish immune response to experimental and natural infection with helminth parasite[J]. *Ann Rev of Fish Dis*, 1996, **6**: 167—177

[13] Harris J E. The immune response of a cyprinid fish to infections of the acanthocephalan *Pomphorhynchus laevis*[J]. *Int J Parasitol*, 1972, **2**: 469—495

[14] Meeusen E N T, Balic A. Do eosinophils have a role in the killing of helminthes parasites?[J]. *Parasitol Today*, 2000, **16**: 95—101

[15] Reite O B. Mast cell/eosinophilic granule cells of salmonids: staining properties and responses to noxious agents[J]. *Fish Shellfish Immunol*, 1997, **7**: 567—584



图版 I

1. 强壮粗体虫吻部伸入到宿主鳜肠壁的固有膜层，在吻部附近的固有膜层观察到白细胞的浸润 Hv：强壮粗体虫；P：吻；E：肠上皮层；Lp：固有膜层；L：白细胞 × 40；2. 虫体寄生部位，固有膜层结缔组织增生，H：吻钩，Ct：结缔组织，×200；3. 虫体吻部与肠壁接触处宿主细胞的种类及分布，靠近虫体吻部依次分布有：纤维细胞、成纤维细胞、嗜酸性粒细胞，Fc：纤维细胞；Fb：成纤维细胞；ECG：嗜酸性粒细胞，× 3000；4. 嗜酸性粒细胞分布在与虫体相接触的肠上皮及固有膜层，部分嗜酸性粒细胞甚至分布到虫体体壁上，Tt：体壁，× 200；5. 在嗜酸性粒细胞、粘液细胞、巨噬细胞及淋巴细胞聚集的地方，虫体已发生脱落或迁移，仅留下寄生过的组织凹痕 Mc：粘液细胞；MØ：巨噬细胞；L：粒细胞；TT：组织凹痕，× 400；6. 未成熟嗜酸性粒细胞，胞质中可观察到三个不同成熟阶段的电子密度颗粒，S1：电子透明颗粒；S2：中等密度电子颗粒；S3：电子致密颗粒；Mt：线粒体；V：微管泡，×12000；7. 成熟嗜酸性粒细胞，胞核位于细胞一侧，胞质中分布有大量电子致密颗粒（星号），×8000；8. 脱颗粒的嗜酸性粒细胞，电子致密颗粒分布于胞质边缘（星号），细胞膜外侧也散布有少量电子密度颗粒（箭头），× 7000；9. 在肠壁的横切面上的固有膜层可观察到正在形成中的结缔组织囊，N：结缔组织纤维囊，× 200；10. 示已形成的具有三层结构的结缔组织囊，Iw：囊内壁；Ow：囊外壁；M：囊中间层，×400；11. 在固有膜层可观察到组织空腔的存在，空腔内有细胞浸润的发生，Tc：组织空腔；Ci：细胞浸润；Lm：肌层，× 350

1. The proboscis of *Hebesomea violentum* in the epithelium of the intestine and the lamina propria with the infiltration of leukocytes. Hv: *Hebesomea violentum*; P: proboscis; E: epithelium; Lp: lamina propria; L: leukocyte, ×200; 2. *Hebesomea violentum* penetrated lamina propria. Note the host response of connective tissue hyperplasia. H: hook; Ct: connective tissue, ×200; 3. The inflammatory cells and their distribution at the host-parasite interface, Fb: fibroblast cell; Fc: fibre cell, × 3000; 4. Eosinophilic granulocyte dispersed in the epithelium and lamina propria. Note some adhereing to the trunk tegument of *Hebesomea violentum*. ECG: eosinophilic granulocyte; Tt: trunk tegument, ×200; 5. Celluar infiltration into the tissue trail where *Hebesomea violentum*

INTESTINAL PATHOLOGY OF THE MANDARIN FISH *SINIPERCA CHUATSI* INFECTED NATURALLY WITH THE ACANTHOCEPHALAN *HEBESOMEA VIOLENTUM*

XIE Hai-Xia, GAO Qian and NIE Pin

(*Institute of Hydrobiology, The Chinese Academy of Sciences; State Key Laboratory of Freshwater Ecology and Biotechnology, Wuhan* 430072)

Abstract: *Siniperca chuatsi* carrying heavy infections of *Hebesomea Violentum* in the gut were obtained from Liangzi Lake, Hubei Province. For light microscopical examination, the gut were fixed overnight in MFAA (Mathanol:Formalin:Acetic acid=85:10:5). After embedding in Histowax, the tissues were serially sectioned at 5μm and stained with May-Grünwald Giemsa using standard techniques. For transmission electronic microscopical examination, small pieces of gut with attached parasites were immersed in ice-cooled fixative and minced in approximately 2mm^3 pieces and then they were fixed in 2.5% glutaraldehyde in phosphate buffered saline (PBS; 0.1 M; pH 7.2) for 4 hours at 4℃ and postfixed for 1h in 1% osmium tetroxide in the same buffer at 4℃, and embedded in Epson-812. Semithin sections were stained with 1% aqueous solution of toluidine blue to select the most suitable areas. Ultrathin sections were double-stained with uranil acetate and lead citrate, and observed in a JEOL JEM-1230 electron microscope at 80kV. The parasite-induced histopathology was related to the penetration of *H. violentum* proboscis in the intestine wall. The host responded to the parasites with connective tissue hyperplasia and with an infiltration of leukocytes, predominantly eosinophilic granulocytes. Eosinophils were accumulated in the epithelium and lamina propria of the infected gut and some adhere to the parasite tegument and proboscis attachment. These eosinophils were subdivided into immature eosinophils, mature eosinophils and degranulating eosinophils. Fibre cells, fibroblast cells, and eosinophils were orderly distributed at the interface of the parasite-host. In addition, *H. violentum* elicits the formation of nodule in tunica propria. These nodules comprised a tri-layered structure to wrap the *H. violentum*. Tissue cava infiltrated by host cells were observed in the lamina propria and they are presumed to be the degenerated nodules.

Key words: Acanthocephalan; *Hebesomea violentum*; *Siniperca chuatsi*; Intestinal pathology

migrated or lost. Note the majority of cells are eosinophilic granulocytes, mucus cell, macrophages and lymphocytes. Mc: mucous cell; MØ: macrophage; Ly: lymphocyte; TT: tissue trail, × 400; 6. Immature eosinophilic granulocyte with granules at different stages of maturation. Granules in stages 1—3 (S1, S2, S3) showing the maturation sequence, Mt: mitochondria; V: vacuoles, × 12000; 7. Mature eosinophilic granulocyte (EGC), with eccentrically located nucleus and large homogeneous electron dense granules (asterisk), × 8000; 8. Degranulation of eosinophilic granulocyte. Note the distribution of the electron-dense granules, most peripherally located inside the cell membrane (asterisk), others dispersed outside the membrane (arrow), × 7000; 9. A partially-formed nodule observed in the lamina propria of the cross-section of the gut. N: nodule, × 200; 10. A well-developed nodule which with a tri-layered structure and *Hebesomea violentum* wrapped by the nodule. Iw: inner wall; M: mesosphere; Ow: outer wall, × 400; 11. Tissue cavum infiltrated by host cells in the lamina propria. Tc: Tissue cavum; Ci: cell infiltration; Lm: lamina muscularis, × 350