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Histopathology of the stomach lesion caused by *Physaloptera brevivaginata* (Nematoda: Physalopteridae) in bats in Spain

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Abstract. *Physaloptera brevivaginata* has been found parasitising the stomach of two species of bats of the family Vespertilionidae, *Myotis myotis* and *Myotis blythii*, in Spain. A comparative study of the prevalences and mean intensities of parasitism by this physalopterid revealed no statistically significant differences between the two hosts. Likewise, no relationship was found between parasite intensity and host body weight. The histopathological study of the stomach lesion revealed destruction of the mucosa, with degeneration of the gastric glands, loss of the *muscularis mucosae* and focal necrosis at the points where the cephalic extremities of both sexes of this nematode attach to the mucosa. The present paper is the first study of gastric pathology caused by an adult physalopterid in bats.

Species of the nematode family Physalopteridae live in the stomach or intestines of all classes of vertebrates. They attach to the mucosa and can cause gastritis, enteritis and excess mucus secretion (Levine 1980). Of the many species included in the genus *Physaloptera* Rudolphi, 1819, bats have been reported as definitive hosts of only four species: *P. retusa* Rudolphi, 1819, *P. brevivaginata* Seurat, 1917, *P. bedfordi* Ortlepp, 1932 and *P. myotis* Babos, 1954. Studies of the helminth fauna in bats in Spain detected *P. brevivaginata* in *Myotis blythii* (Tomes, 1857) (see Esteban et al. 1995), the worldwide spectrum of hosts being extended in this work to include *M. myotis* (Borkhausen, 1797). In the present paper we compare the prevalence, intensity and abundance index (Pence and Eason 1980) of infection by *P. brevivaginata* in the two host species. This is followed by a description of the histopathology of the stomach lesions found in both bat species, which have already been the subject of a preliminary study (see Esteban et al. 1991).

MATERIALS AND METHODS

During 1984–1993, a total of 31 specimens of *Myotis myotis* (*M. m.*) and 34 specimens of *M. blythii* (*M. b.*) were trapped and killed by chiropteran mammalogists. This material originated from several discrete populations of both hosts. Three of the bats, specifically those that posteriorly exhibited stomach perforation, showed very little activity and were captured in consequence. The material was obtained in 5 different geographic locations: Teruel (2 *M. b.*), Barcelona (4 *M. m.* and 3 *M. b.*), Lérida (3 *M. m.*), Huesca (1 *M. b.*) and Valencia (24 *M. m.* and 28 *M. b.*). Only 13 specimens (7 *M. m.* and 6 *M. b.*) coming from Valencia were infected by *P. brevivaginata*.

The parasitized stomachs were removed and opened longitudinally and washed several times with saline solution, before fixation in Bouin solution supplemented to 1% in urea. The stomachs were dehydrated, embedded and sectioned according to routine histological practice. Sections, 5–10 μm thick, were stained with Harris' hematoxylin and eosin and Masson's trichromic stain.

Nematode specimens and histological sections of the stomachs were deposited in the Department of Parasitology, Faculty of Pharmacy, University of Valencia (Valencia, Spain).

RESULTS

Parasitological results

The study of the total *Myotis* specimens analysed allowed us to detect a helminth community, identical in both hosts species, comprising 7 species: *Plagiorchis* (*Plagiorchis*) sp., *Prosthodendrium* (*Prosthodendrium*) *corberensis* Esteban et al., 1991, Gyrabascinae gen. sp. (Trematoda), *Hymenolepis grisea* (Beneden, 1873 (Cestoda), *Aonchotheca moraveci* Esteban et al., 1991, *Molinostrongylus alatus* (Ortlepp, 1932) and *Physaloptera brevivaginata* (Nematoda). Sixty-four specimens (11 males, 10 mature females and 43 immature females) and 139 specimens (67 males, 55 mature females and 17 immature females) of *P. brevivaginata* were collected

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from 31 and 34 individuals of *M. m.* and *M. b.*, respectively. Both bat species are common hosts of *P. brevivaginata*, as reflected by the abundance indices (A) obtained (always greater than 1 : *M. m.* : A = 2.06; *M. b.* : A = 4.01; maturity factor = 1). This shows that this physalopterid constitutes a dominant species in the helminth fauna of these host species (Pence and Eason 1980).

There was no significant difference in prevalence of infection by *P. brevivaginata* in the two hosts (*M. m.* = 22.6%; *M. b.* = 17.6%) (*Chi*-square test , $X^2 = 0.274$, 1 df, $p < 0.05$). Likewise, the differences observed in the mean intensities of infection (*M. m.*: 9.1 ± 5.2; *M. b.* : 23.2 ± 25.2; mean ± SD) were not significant (*t*-test, following the logarithmic transformation of the Intensity values – y = log I - for fitting the data to a normal distribution, t = 1.687, 11 df, $p < 0.05$) (see Sokal and Rohlf 1969). The infection intensity range was 1–43 in *M. myotis* and 3–78 in *M. blythii.* As all the hosts analysed were adults, the correlation between the number of *P. brevivaginata* in each host (following the logarithmic transformation of the Intensity values – y = log I - for fitting the data to a normal distribution) and host weight was nonsignificant (linear correlation coefficient, r = 0.037 and r = 0.611 for *M. m.* and *M. b.*, respectively).

Histopathological results

Infection by *P. brevivaginata* is clearly apparent when the abdomen of the host is opened. A small, irregular and intensely red spot with a whitish-yellow aureola corresponding to the point where the nematode attaches to the mucosa is usually visible on the serosal aspect of the stomach. Usually a single attachment point is seen, and only sporadically are two or three such points found. The males and females of the parasite, frequently copulating, cause a V-shaped cratered lesion. No more than a dozen specimens are usually found in each lesion, though in two hosts we detected totals of 29 and 78 - these individuals being attached to the mucosa at the same point and causing extensive pathology. The size of the cratered lesions exhibited a certain proportionality related to burdens present, with the exception of one case presenting only immature females, in which no stomach lesion were observed. In addition, in three cases there was complete perforation of the gastric wall, producing an ulcer of considerable size. In one of these cases a single gravid female had passed through to attach to the peritoneum. This caused a lesion of similar appearance to those seen in the stomach, no local peritonitis being observed. No differences were detected between the lesions in the two host species. Beyond the contours of the pathology caused by *P. brevivaginata*, the appearance of the host stomach wall is normal, and corresponds to the basic histological pattern of many mammals in general, and of insectivorous bats in particular (see Rouk and Glass, 1970). The mucosa shows prominent folds in which the submucosa, muscle layer and serosa appear uniform, without alterations (Fig. 1A). At the contours of the lesion (periulcer zone) the mucosa exhibits a normal architecture, with an intact epithelium (Fig. 1B). The submucosa presents an intense oedema, with dilatated capillaries and prominent endothelia. An inflammatory infiltrate is observed consisting of polymorphonuclear and mononuclear cells, including macrophages, with proliferation of fibroblasts, along with diffuse red blood cells that do not form hemorrhagic foci. The muscle layer and serosa show no alterations. In the region of the crater lesion (ulcerated zone) the mucosa is found to be highly altered, with a total loss of the gastric glands and destruction of the *muscularis mucosae* (Fig. 1C). In the zone where the nematode penetrates, the mucosa presents epithelial ulceration, with an inflammatory infiltrate composed of polymorphonuclear cells, mast cells, plasma cells, macrophages, fibroblasts and diffuse red blood cells. Abundant cellular debris is also seen. The parasites attach by their cephalic ends at the limit between mucosa and submucosa. These cephalic ends are lodged within an area of dense collagen fibrosis extending as a reticle through the muscle layer, interrupting the latter and exerting discrete traction on the serosa at this level (Fig. 1D). The submucosa shows an inflammatory infiltrate of the same characteristics as that found in the mucosa.

DISCUSSION

Species of the genus *Physaloptera* occur in the stomach of a variety of terrestrial vertebrates. Although it is unknown for *P. brevivaginata*, the life cycles of several species of the same genus have been studied, i.e., *P. turgida* Rudolphi, 1819, *P. retusa* Rudolphi, 1819, *P. maxillaris* Molin, 1860, *P. praeputialis* Linstow, 1889, *P. dispar* Linstow, 1904, *P. rara* Hall et Wigdor, 1918 and *P. hispida* Schell, 1950 (see Seurat 1916, Alicata 1937, Hobmaier 1941, Basir 1948, Petri 1950, Petri and Ameel 1950, Schell 1952, Zago Filho 1959, Lincoln and Anderson 1975, Cawthorn and Anderson 1976, Seureau and Quentin 1977, 1986). In all cases the cycles are diheteroxenous and mediated by insects. Thus, insects scavenging fecal material ingest eggs which hatch in their gut and then migrate into body tissues for subsequent development to third stage larvae. These larvae are in turn infective to definitive hosts. Nevertheless, some *Physaloptera* spp. have been found encysted in certain vertebrates (see Dixon and Roberson 1967, Widmer 1970), though very probably these are paratenic hosts. The presence of *P. brevivaginata* in *M. myotis* and *M. blythii* agrees with the results

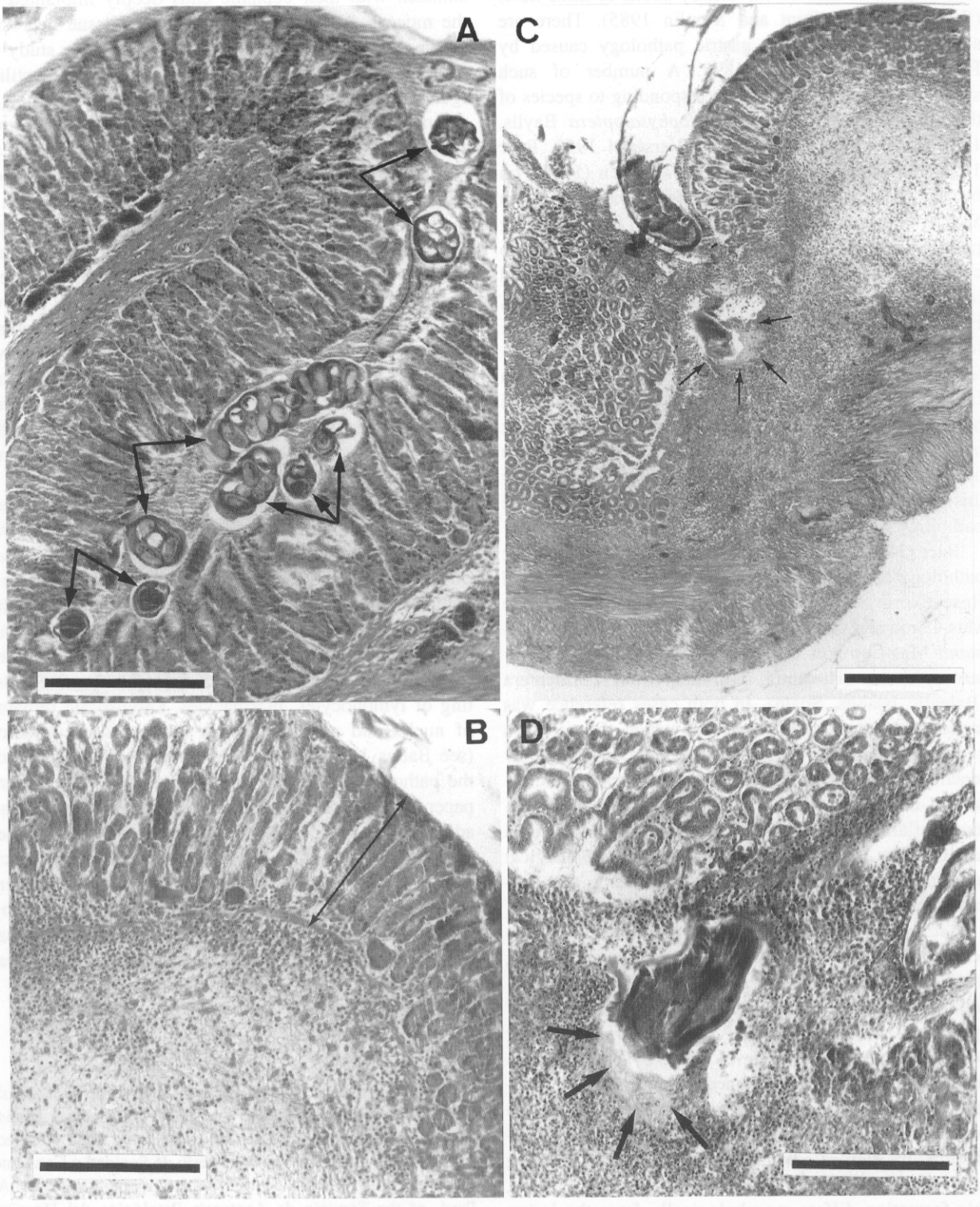

Fig. 1. Histopathology of the gastric lesion produced by *Physaloptera brevivaginata* in *Myotis blythii*. **A** – appearance of the healthy stomach folds, between which sections of various specimens are observed (arrows); **B** – submucosal hyperplasia with an abundant inflammatory infiltrate in the vicinity of the lesion (periulcer zone); the glandular epithelium is intact (arrow); **C** – ulcer crater formed by the parasite at the point where it attaches to the mucosa; there is complete loss of the gastric glands, with destruction of the *muscularis mucosae*; nematode attaches to the wall at submucosal level (arrows); **D** – detail of the insertion of the cephalic extremity of the nematode in the submucosa, causing tissue necrosis (arrows) and an abundant inflammatory response. Scale bars: A, B, D: 230 μm; C: 360 μm.

accumulated to date on the dietary habits of these hosts (Bauerova 1984, Pont and Moulin 1985). There are some references to host gastric pathology caused by Physalopterinae Railliet, 1893. A number of such studies have been published corresponding to species of the closely related genus *Pseudophysaloptera* Baylis, 1934 (Chen 1937, Kobulej and Versenyi 1953, Baer 1959, Mas-Coma and Gállego 1977). Chen (1937), on occasion of the finding of *P. soricina* Baylis, 1934 in *Suncus coerulus* (Insectivora: Soricidae) in Kwangtung province (South China), schematically represented two copulating specimens with their extremities anchored in the mucosa, in clearly separated areas (Chen 1937, Fig. 10). Neither the drawing nor the text made any reference to any pathological formation, however. Kobulej and Versenyi (1953), in their work on the helminth fauna of soricids from Hungary, described *P. kotlani* based on material from *Crocidura leucodon* and *Sorex araneus* (Insectivora: Soricidae). They described the nodule-form deformation of the stomach wall caused by this species, though no accompanying illustration of the pathology was included.

Baer (1959) gave a detailed description of the gastric pathology caused by *P. soricina* in *Scutisorex somereni* (Insectivora: Soricidae) from the Belgian Congo, while Mas-Coma and Gállego (1977) did the same for *P. kahmanii* Mas-Coma et Gállego, 1977, in *Eliomys quercinus ophiusae* (Rodentia: Gliridae) from Formentera (Balearic Islands, Spain). In both cases reference was made to a torus-shaped crater in which the parasites cause complete destruction of the mucosa and may reach the submucosa, where the tissue appears necrotized at the points where the nematodes anchor. An intense inflammatory reaction is observed (see Mas-Coma and Gállego 1977, Fig. 3). Thus, from the histological point of view these pathologies are similar to that described in the present study.

As regards *Physaloptera* spp., Seurat (1937) described the pathology produced by *P. getula* Seurat, 1917 in a specimen of *Meriones shawi* (Rodentia: Gerbillidae) from the North of Africa. This author found 7 specimens implanted in the stomach wall by their cephalic extremity, at the level of the pylorus. This nematode produces a dome-shaped lesion that exhibits intense mucosal hyperplasia, with destruction of the *muscularis mucosae* and a great agglomeration of inflammatory elements within the submucosa. According to the illustration provided by this author (Seurat 1937), this formation differs morphologically from the lesion described in our study, though it is histologically perfectly comparable. Pearce and Tanner (1973) described infection by *P. retusa* Rudolphi, 1819, of *Sceloporus magister*, *S. occidentalis* and *S. undulatus* (Reptilia: Iguanidae) from Utah (U.S.A.). They described the presence of these nematodes in the proximal third of the stomach with their cephalic ends deeply implanted in the mucosa. No mention was made of tissue necrosis. Goldberg and Bursey (1989), in an exhaustive study of infection by *P. retusa* of *Sceloporus graciosus* (Reptilia: Iguanidae) from California (U.S.A.), reported that in recent U-shaped lesions the nematodes caused an erosion of the surface epithelium which reached as deep as *muscularis mucosae* and resulted in up to total loss of gastric glands at the region of attachment. Likewise, a marked mixed inflammatory response is observed, with necrosis at the points where the cephalic ends attach (see Goldberg and Bursey 1989). In addition, the oldest lesions showed fibrous scars corresponding to the regenerative process of the gastric wall (see Goldberg and Bursey 1989). According to these authors, the erosive action of *P. retusa* in *S. graciosus* is more superficial than that described in our study, for parasites of reptiles barely reached the *muscularis mucosae*; moreover, the lesions are clearly recoverable and in no case is there complete perforation of the stomach wall.

Finally, Babos (1954) carried out a brief histological study of the nodules caused by larvae of *P. myotis* Babos, 1954 in the stomach mucosa of *Myotis oxygnathus* (Chiroptera: Vespertilionidae) from Hungary. This author reported that the nodules were situated in the *muscularis mucosae*, with no signs of acute inflammation. In addition, the transverse section of the nodules showed the larve to be embedded in a soft mass consisting of lymphocytes, plasmacytes, karyosomic remains of nuclei and a homogeneous eosinophilic substance (see Babos 1954). However, no mention was made of the pathology caused by adult specimens. The present paper is the first study of gastric pathology caused by an adult physalopterid in bats. This pathology may be severe enough to cause the death of the host species, such as in a case of gastric perforation as a sequel to ulceration. This fact could confirm the idea proposed by Esteban et al. (1991) regarding the possible role that helminths may occasionally play as regulators of bat populations.

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