Stomach wall changes caused by the presence of third-stage *Anisakis simplex* B larvae in infected dogs

Magdalena Podolska[1], Wojciech Piusiński[2] and Jerzy Rokicki[1]

[1]Department of Invertebrate Zoology, University of Gdańsk, Piłsudskiego 46, 81-378 Gdynia; [2]Department of Pathology, Veterinary Faculty, Agriculture University, Grochowska 272, 03-849 Warszawa; Poland

Abstract. Samples of stomach fundus and pylorus tissue collected from eight dogs experimentally infected with third-stage *A. simplex* B larvae were examined macro- and microscopically. The histopathological changes in these samples were analysed. Pathomorphological examination revealed that *A. simplex* B larvae actively penetrated the dogs stomach wall within 2 days of infection. The presence of larvae in the mucous membrane was accompanied by tissue damage and cell infiltration predominantly by eosinophils.

Introduction

Ascaroid nematodes that mature in marine mammals are a source of pathology and pathogenesis in their hosts. The ulcers associated with larvae *Anisakis simplex* B in the forestomach of harbour porpoises *Phocoena phocoena* (L.) from the North Sea were described by Smith (1989). Porpoises from the Baltic Sea did not display any signs of ulcers caused by penetration of larvae, and the presence of *A. simplex* was not detected (Rokicki et al. 1997); however, mature *A. simplex* were found in one of four white-beaked dolphins *Lagenorhynchus albirostris* examined, its stomach carrying the scars caused by ulceration (Skóra et al., in press).

Man, along with a number of terrestrial mammals, is an accidental host of these nematodes. Anisakiosis of the alimentary canal may be the cause of a number fairly dangerous complications (Van Thiel et al. 1960), and can even bring about the onset of some forms of stomach cancer (Petithory et al. 1990). Other investigations (Van Thiel et al. 1960, Myers 1963, Morishita and Nishimura 1965, Otsuru et al. 1965, Ishikura et al. 1967, Van Thiel and Van Houten 1967, Yokogawa and Yoshimura 1967, Tanaka et al. 1968, Kim et al. 1971, Tanabe et al. 1990), dealing mainly with the pathogenicity of *Anisakis* larvae, were carried out in regions other than the Baltic. *A. simplex* has a world-wide distribution, occurring in all major oceans and seas. On the basis of electrophoretic studies, Mattiucci and Paggi (1989) demonstrated that two sibling species are included within *A. simplex* A and *A. simplex* B. Most specimens of *A. simplex* A occur in the Mediterranean Sea, whereas *A. simplex* B is found mainly in the North Atlantic. Specimens of the two forms can also coexist in the same area and even in the same host (Nascetti et al. 1986). In the Baltic Sea, only *A. simplex* B has been noted (Mattiucci et al. 1989). The aim of the present study was to estimate the invasion capabilities of third-stage *A. simplex* B larvae occurring in herrings *Clupea harengus membras* in the southern Baltic, and to assess the nature of the damage these nematodes inflict on the alimentary canal of accidental hosts. So far, no such investigations using *A. simplex* B larvae for the experimental infection of dogs have been conducted.

Materials and methods

The *A. simplex* B larvae collected from Baltic herrings *C. harengus membras* were kept up to 48 h in 0.9 % NaCl solution. Eight dogs were experimentally infected per os with 50 specimens of third-stage *A. simplex* larvae. The dogs were autopsied 1, 2, 3, 4, 5, 6, 7 and

8 days following infection. Tissue samples, collected from the fundus and pylorus of the dogs stomachs containing parasites, were fixed in 10% formaldehyde. They were then washed in tap water, dehydrated in an ethanol series, cleared in xylene, embedded in paraffin wax and sectioned. The 4 to 5 m thick tissue sections were stained with haematoxylin and eosin.

Results

Pathomorphological examinations

Macroscopic examination of a dogs stomach, excised and dissected 2 days after infection, revealed the presence of *A. simplex* larvae loosely attached to the walls of the fundus and pylorus (Fig. 1). They fell off
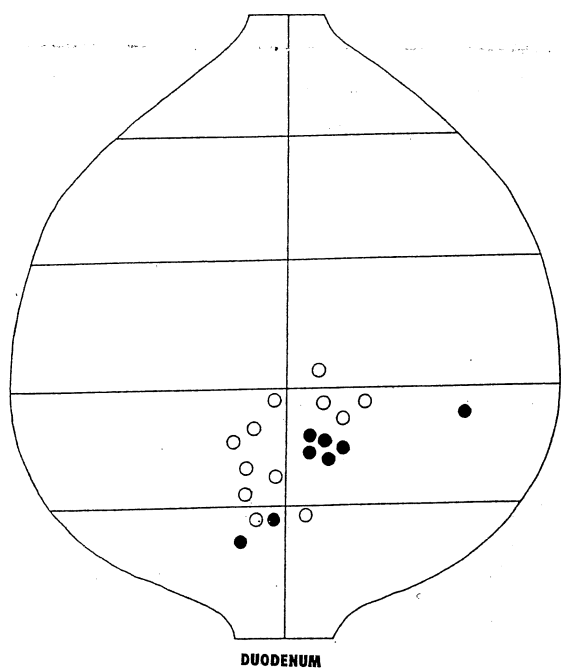

Fig. 1. Schema of the stomach of dogs showing distribution of *A. simplex* larvae; black circles – 2 days after infection, empty circles – 3 days after infection

easily, particularly when placed in the fixing liquid (formaldehyde). Histopathological examination of sections from the parasite-damaged areas showed the following changes: (1) the disappearance of the characteristic gastric pits (foveolae gastrice) in the mucosa; (2) the surface epithelium had become squamous or cuboidal in shape; (3) the mucous coating was absent at these sites. Furthermore, in the mucous membrane

proper, lesions covered much of and sometimes the entire lamina propria of the mucosa right down to but not beyond the muscularis mucosae, forming distinct pits with irregular, frequently necrosed edges in the surface of the mucosa (Fig. 2). Gastric glands or cell fragments were visible in the deeper tissue. Sites adjacent to the damaged tissue were affected by inflammatory infiltration consisting of neutrophils, single macrophages and eosinophils as well as mucosal mast cells. The lesions in the mucous membrane took the form of oval cavities in the stomach wall, lined with some homogeneous substance pink stained with hematoxylin-eosin (Fig. 2).

Macroscopic examination of a dogs stomach examined 3 days after infection, showed *A. simplex* larvae anchored in the stomach wall mucosa. Histopathological examination of stomach wall fragments collected from the infected areas revealed changes due to larval penetration. In some areas, the lesions in the mucosa of the epithelium proper were different in size and depth from those in the stomach wall in the dog examined 2 days after infection. Apart from these lesions, there was evidence of damage in the remaining tissue adjacent to them. The infiltration of neutrophils, macrophages and eosinophils, were also noted. In the parts of the stomach lumen adjacent to the affected areas, the larvae were visible only in cross-section, and occurred at the same depth as the damage to the mucous membrane. At a few sites larvae were found attached to the damaged stomach wall. Parasite penetration caused lesions extending as deep as the muscularis mucosae (Figs. 3 and 4). The lesions were of different shapes and sizes, and sometimes took the form of a burrow with the larva inside. Both sides of this burrow consisted of mucous membrane proper obviously inflamed but otherwise unaffected (Fig. 4). At another site the lesion in the mucous membrane was clearly visible both at the point of entry and its immediate surroundings. In this case the mucous membrane proper on one side of the penetrating larva remained intact though evidently inflamed (Fig. 3). In some areas the tissues surrounding the nematodes were found to be necrosed.

In some parts of the stomach wall of the dog 3 days post infection, larvae had penetrated right down into the submucosa. The presence of larvae there (Figs. 5 and 6) was accompanied by tissue damage and extensive cell infiltration, predominantly by eosinophils (Fig. 7). Infiltration of this kind also occurred in the vicinity of the blood vessels (Figs. 7 and 8). Macrophages and neutrophils were noted in the cell infiltration next to the damaged tissue. Eosinophil infiltration into the submucous membrane was much more pronounced than in the mucous membrane proper.

Mononuclear cells containing eosinophilic granulation were noted (Fig. 10) in the mucous membrane
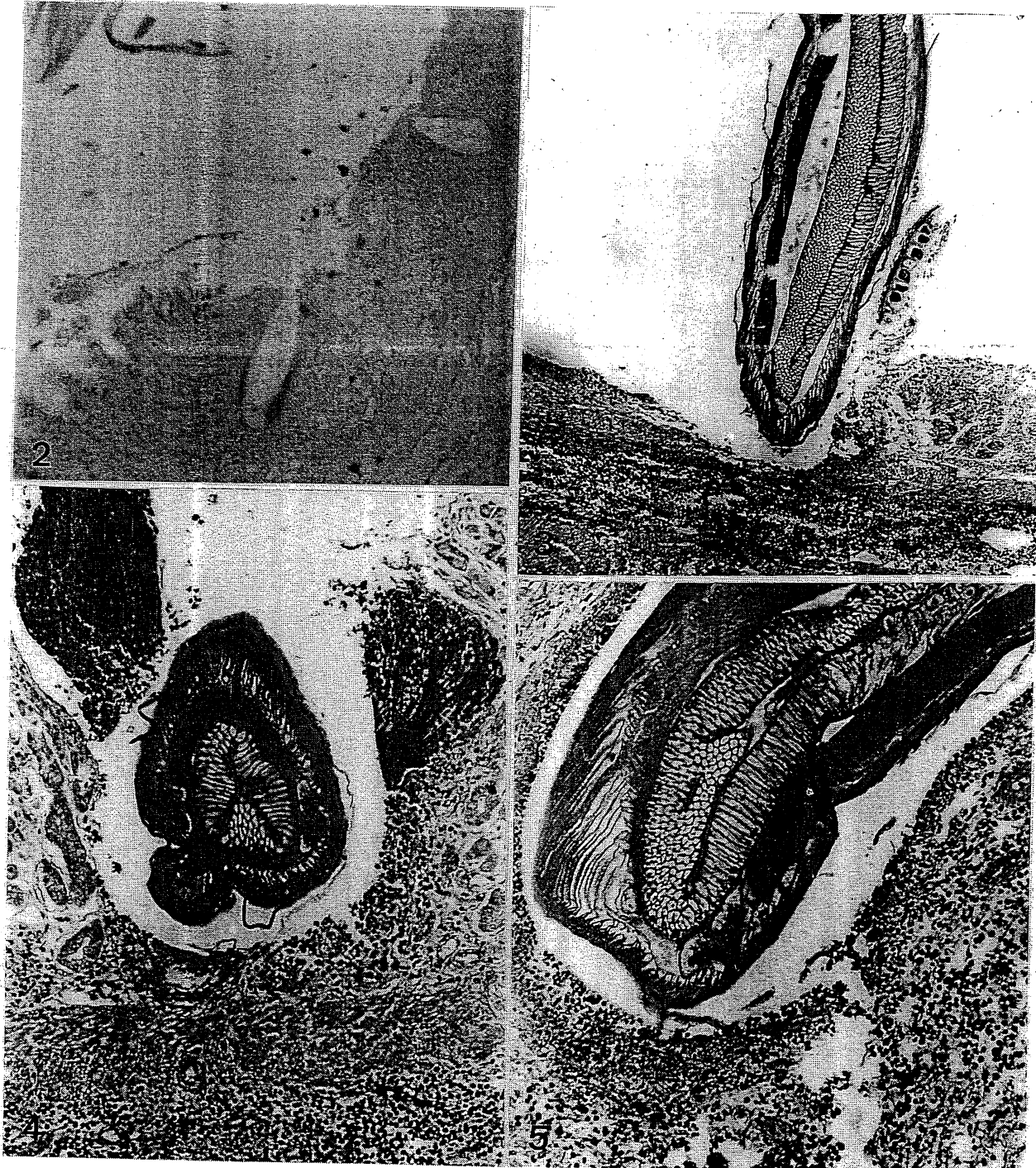
Fig. 2. Mucous membrane from a dogs stomach 2 days after infection. Left-side – intact mucosa with distinct gastric pits; right-side – obvious extensive lesions in the mucosa with two cavities surrounded by homogenous, eosinophilic walls. Near the damaged tissue a cell reaction can be seen. In the upper part of the stomach lumen, there is a fragment of sectioned larva. **Fig. 3.** Larva penetrating a dogs stomach wall 3 days after infection. At the penetration site there is a mucosal lesion with obvious infiltration of cells around the damage. To the left of the penetrating larva is damaged mucosa, to the right is intact mucosa. **Fig. 4.** Cross-section of a larva penetrating the mucosa of a dogs stomach 3 days after infection. On both sides of and below the lesion, a cell reaction and fragments of gastric gland can be seen. **Fig. 5.** Dogs stomach wall 3 days after infection. Larvae penetrating down into the mucosa caused tissue damage and a very pronounced cell reaction
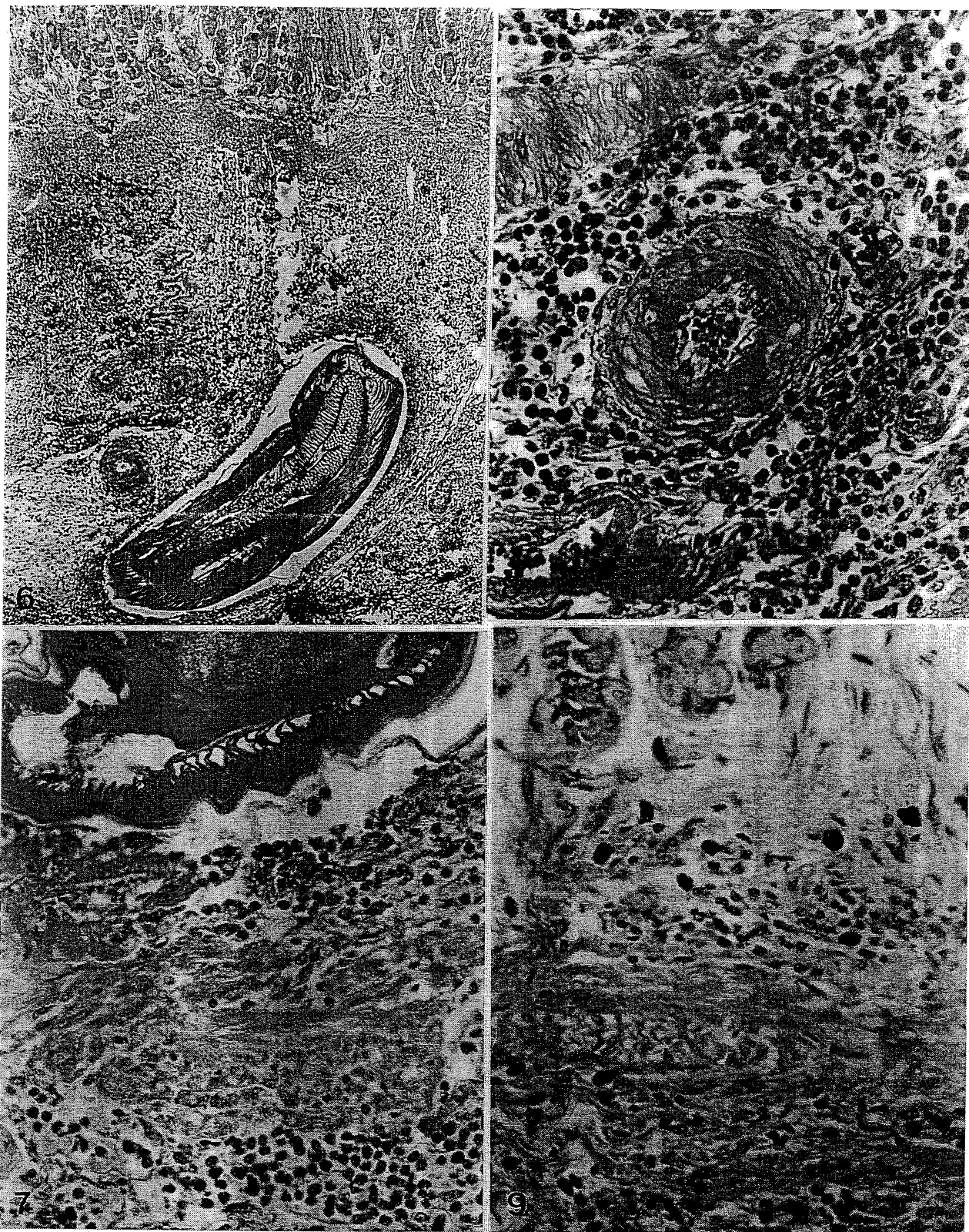
Fig. 6. Fragment of sectioned larva in the submucosa of a dogs stomach 3 days after infection. Surrounding the larva there is a pronounced cell reaction dominated by eosinophils. In the upper part are gastric glands. **Fig. 7.** Stomach wall of a dog 3 days after infection. In the upper part is a fragment of sectioned parasite in the submucosa. In the lower and central parts of the photograph is a large cell infiltration, composed mainly of eosinophils. **Fig. 8.** Stomach submucosa 3 days after infection: infiltration of eosinophils near the larval penetration sites, very numerous eosinophils in the vicinity of blood vessels. **Fig. 9.** Stomach wall 3 days after infection. In the upper part of the photograph fragments of gastric glands are visible. Below, in the subglandular lamina, there are large, mononuclear cells – globule leukocytes (GL). There are numerous eosinophils in the lower part

Fig. 10. Globule leukocytes (GL) present in the stomach mucosa 3 days after infection. These cells contain round or oval nuclei and extensive eosinophilic granulation in the cytoplasm

proper, in the connective tissue and in the lamina subglandularis (Fig. 9). Morphologically, the cells were similar to globule leukocytes (GL) which frequently occur in the epithelium and mucous membrane of vertebrate species, including man. They also occurred quite frequently in the mucous membrane proper, around the damaged tissue and the mucous membrane lesions.

Four days after infection, free 4th-stage larvae were found in the stomach lumen. In the material subsequently taken from the dogs stomach, there were no changes indicative of larval penetration.

Discussion

The results of the present study revealed the presence of *A. simplex* B larvae in the stomach wall of the experimental dogs after 2 and 3 days. After 3 days, the larvae had penetrated the stomach wall much deeper and the pathological changes were more pronounced than after 2 days. Similar results were obtained by Hirabayashi (1972) in dogs infected with *A. simplex* larvae: after having administered 100 nematodes, the author found 50, 26 and 23 larvae in the stomach, intestine and body cavity after two days. Asami et al. (1965) and Oyanagi (1967) also examined dogs infected with *Anisakis* larvae: 12 h later larvae were found anchored in the mucous membrane of the stomach. In other investigations into the experimental infection in different species of animals, larval penetration took place earlier and different sites were affected by pathological changes. In rats experimentally infected with *Anisakis* larvae, the parasites penetrated the stomach wall after 1 h. Free nematodes were found as soon as 3–4 h later in the peritoneum (Young and Lowe 1969, Gibson 1970). Oishi et al. (1969) found that the larvae had been able to migrate through a rats stomach wall within 24 h after infection. According to Young and Lowe (1969), the parasites survived no longer than 7 days after infection. In experimentally infected rabbits, the larvae penetrated the submucous wall of the stomach within 24 h. Very few larvae were attached to the intestinal wall (Kuipers 1964). Myers (1963) demonstrated that larvae administered to guinea pigs penetrated the stomach wall and migrated through the gut, only to be found alive within 5 days. After 6 days only free, 4th-stage larvae were found in the stomach lumen. Fragments of cuticle, cast off by larvae during moulting were attached to the central part of the lesion in the stomach mucous membrane. This hypothesis coincides with the macroscopic detection of larvae, loosely attached to the stomach mucosa at the time of infection. The gastric mucosa showed signs of mechanical damage, and the surrounding tissue had reacted to the presence of these parasitic larvae.

Fujino et al. (1984) removed several 4th-stage *A. simplex* larvae from a patients mucous membrane and suggested that moulting from stage L3 to L4 takes place in the stomach provided that the larvae remain there for 3–5 days. The present studies have confirmed this hypothesis, as it was found that *A. simplex* B larvae in the stomach wall moult and tend to be encountered in the stomach lumen in stage L4.

In marine mammals, the final hosts of *A. simplex*, larval moult and transition from L3 to L4 proceeds in the stomach wall, but the subsequent moult, leading to larval maturation, takes place in the stomach lumen (Kikuchi et al. 1967). Fragments of cuticle cast off by *Anisakis* larvae were found in the centres of stomach wall ulceration in the porpoise *P. phocoena* (Smith 1989). Similar cuticle was observed in present experiment (Fig. 2).

Using a gastroscope to examine anisakiosis in man, Yokogawa and Yoshimura (1967) found the stomach to be the principal site of pathological changes. The present experiments on infected dogs confirm this conclusion.

However, the location of *Anisakis* larvae in the human gastrointestinal tract was different, Van Thiel et al. (1960) found larvae mainly in the intestines of surgically operated patients. Myers (1963) found parasites in the wall of the stomach, small intestine, the pancreas, the perirenal tissue and even in the thyroid

gland of guinea pigs. *Anisakis* larvae were also found on the peritoneum (Ishikura et al. 1967, Van Thiel and Van Houten 1967). These parasites can be found in many other sites in the alimentary canal, such as the caecum, colon and rectum, and also away from the intestine – in the lymph nodes, pancreas, reticulum and mesentery (Otsuru et al. 1965, Van Thiel and Van Houten 1967, Yokogawa and Yoshimura 1967). In single cases, the larvae penetrated the throat wall (Morishita and Nishimura 1965, Tanaka et al. 1968, Kim et al. 1971) and even the tongue (Tanabe et al. 1990). The present paper comments only on changes to the stomach wall resulting from larval penetration.

One of the characteristic pathological changes caused by *Anisakis* larvae that occur in the hosts alimentary canal wall is eosinophilia in the tissues surrounding the parasite (Van Thiel et al. 1960, Young and Lowe 1969, Gibson 1970, Oshima 1972). This was confirmed by the present study. Young and Lowe (1969) and Gibson (1970) noted a slight infiltration of eosinophils around a larva that had penetrated the entire intestinal wall.

Van Thiel et al. (1960) describe an inflammatory infiltration in all the layers of the intestinal wall in man. It was purulent, containing neutrophils.

Jackson et al. (1967) examined the pathology of experimental anisakiosis in miniature pigs. The majority of the larvae were in the submucous membrane.

This diversity of results is due mainly to the animal species used in the experiments. In small animals, which have thin stomach walls, the parasites penetrate the mucous membrane sooner and break through into the peritoneum. The extent of infiltration by *Anisakis* larvae therefore depends largely on the structure and thickness of the stomach wall. Other factors must also be taken into account: the interindividual sensitivity which probably occurs between the animals, and their condition, as well as differences in the infection capability of the parasites and their number. Using an electrophoretic technique, Nascetti et al. (1983, 1986), Orecchia et al. (1983), Paggi et al. (1983), Mattiucci and Paggi (1989), Mattiucci et al. (1989) recognised two sibling species of *A. simplex*, differing in enzyme composition. Both species, denoted by these authors as *A. simplex* A and *A. simplex* B, are morphologically related to the type of larvae described by Berland (1961) and Oshima (1972). The Japanese authors may have been using *A. simplex* A larvae. In some cases, the species of larvae was not stated, merely described as *Anisakis* sp.; they could conceivably have been *A. physeteris* or *A. typica*. In the present study, the larvae used for the infection were collected from the southern Baltic, a region where only *A. simplex* B larvae have been reported so far (Mattiucci et al. 1989).

The *Anisakis* larvae used in the experiments of other authors were derived from different fish species in distant geographical regions. The present study was based only on nematodes derived from the Baltic herring *C. harengus membras*.

The infection capability of the parasites is largely dependent on the time span covering the collection of larvae, the infection of the host animal, the parasite preserving techniques and the possibility of mechanical damage to the larvae. Those used by Gibson (1970) for infecting rats had been preserved in NaCl solution up to 48 h before they were administered to the animals (the present study followed the same routine). Asami and Inoshita (1967) stated that although 75% of nematodes remain motile after 20 days of preservation at 4°C, this procedure renders them incapable of infecting guinea pigs.

American and Japanese authors have described the pathological changes caused by *Anisakis* larvae in pigs. Ashizawa et al. (1973a, b) and Usui et al. (1973) described 3 types of damage occurring in pig stomachs as a result of *Anisakis* larvae infection due to the consumption of fish by-products.

The histopathological changes observed in the mucous membrane of the stomach in infected dogs in the present study demonstrated that, apart from mechanical damage characteristic of type I according to Ashizawa et al. (1973a, b) and Usui et al. (1973), the presence of parasites caused blood vessel rupture and extravasation which, in turn, resulted in oedema, inflammatory infiltration and local necrosis. Oshima (1972) classified *Anisakis* larvae-related damage according to the state of the lesions. A foreign-body-response was described by this author from the histopathologic point of view as a neutrophilic and eosinophilic infiltration. A small oedema, a fibrinous exudate, a haemorrhage, and blood vessel damage should be taken into account. An eosinophilic granuloma is likely to be formed around a larva. The present results confirm the occurrence of inflammatory infiltration around a larva which has not yet become an eosinophilic granuloma. However, the changes described here refer to a fairly early period following infection, and the possibility of eosinophilic granuloma, frequently occurring in more advanced cases of anisakiosis, cannot be ruled out (Oshima 1972). The mucous membrane lesions encountered could lead to later ulcerations. The changes noted during the present study resemble the foreign-body-response described by Oshima (1972).

Globule leukocytes (GL), which appeared near the damaged tissue, are regarded as transformed mucosal mast cells (Akpavie and Pirie 1989). Murray et al. (1968) proved the relationship between parasitic infection and GL occurrence. Huntley et al. (1984) stated that the latter is an immunological response to parasitic

infection. GL cell role and mechanisms of its action are currently under discussion (Akpavie and Pirie 1989).

References

Akpavie S. O., Pirie H. M. 1989. The globule leucocyte: morphology, origin, function and fate, a review. *Anatomia, Histologia, Embryologia*, 18, 87–95.

Asami K., Inoshita Y. 1967. Experimental anisakiasis in guinea pigs: Factors influencing infection of larvae in the host. *Japanese Journal of Parasitology*, 16, 415–422 (in Japanese).

Asami K., Watanuki T., Sakai H., Imano H., Okamoto R. 1965. Two cases of stomach granuloma caused by *Anisakis*-like larvae nematodes in Japan. *American Journal of Tropical Medicine and Hygiene*, 14, 119–123.

Ashizawa H., Nosaka D., Tateyama S., Usui M. 1973a. Studies on swine gastric anisakiasis. II. Macroscopic lesions. *Bulletin of the Faculty of Agriculture. Miyazaki University*, 20, 179–189 (in Japanese).

Ashizawa H., Nosaka D., Tateyama S., Usui M. 1973b. Studies on swine gastric anisakiasis. III. Histopathological findings. *Bulletin of the Faculty of Agriculture. Miyazaki University*, 20, 191–201 (in Japanese).

Berland B. 1961. Nematodes from some Norwegian marine fishes. *Sarsia*, 2, 1–50.

Fujino T., Ooiwa T., Ishii Y. 1984. Clinical, epidemiological and morphological studies on 150 cases of acute gastric anisakiasis in Fukoka Prefecture. *Japanese Journal of Parasitogy*, 33, 73–92 (in Japanese).

Gibson D. I. 1970. Aspects of the development of "herringworm" (*Anisakis* sp. larva) in experimentally infected rats. *Nytt Magasin for Zoologi*, 18, 175–187.

Hirabayashi H. 1972. Comparative study on the pathogenesis of *Anisakis* type-I larvae from several sea fishes obtained different areas of Japan. *Yokohama Medical Journal*, 23, 405–418 (in Japanese).

Huntley J. F., Newlands G., Miller H. R. P. 1984. The isolation and characterisation of globule leucocytes: Their derivation from mucosal mast cells in parasitised sheep. *Parasite Immunology*, 6, 371–390.

Ishikura H., Hayasaka H., Kikuchi Y. 1967. Acute regional ileitis at Iwanai in Hokkaido with special reference to intestinal anisakiasis. *Sapporo Medical Journal*, 32, 183–196.

Jackson G. J., Bier J. W., Payne W. L. 1967. Experimental anisakiasis in pigs: course of infection with larval *Anisakis* sp. and *Phocanema* sp. nematodes from fishes. *Transactions of the American Microscopical Society*, 95, 264 (Abstract).

Kikuchi Y., Ueda T., Yoshiki T., Aizawa M., Ishikura 1967. Experimental studies of the immunopathology of the intestinal anisakiasis. *Igakuno Ayumi*, 62, 731–736 (in Japanese).

Kim C. H., Chung B. S., Moon Y. I., Chun S. H. 1971. A case report on human infection with *Anisakis* sp. in Korea. *Korean Journal of Parasitology*, 9, 39–45 (in Korean).

Kuipers F. C. 1964. Pathogenese van de harinwormflegmone bij de mens. *Nederlands Tijdschrift voor Geneeskunde*, 108, 304–305.

Mattiucci S., Paggi L. 1989. Multilocus electrophoresis for the identification of larval *Anisakis simplex* A and B and *Pseudoterranova decipiens* A, B and C from fish. In: *Nematode problems in North Atlantic fish* (Ed. H. Möller). ICES CM/F, 6, 23.

Mattiucci S., D'Amelio S., Rokicki J. 1989. Electrophoretic identification of *Anisakis* sp. larvae (Ascaridida: Anisakidae) from *Clupea harengus* L. in Baltic Sea. *Parassitologia*, 31, 45–49.

Morishita K., Nishimura T. 1965. Studies on the *Anisakis* larvae (2). *Anisakis* larva from human buccal mucosa. *Japanese Journal of Parasitology*, 14, 54 (in Japanese).

Murray M., Miller H. R. P., Jarret W. F. H. 1968. The globule leucocytes and its derivation from the subepithelial mast cell. *Laboratory Investigation*, 2, 222–234.

Myers B. J. 1963. The migration of *Anisakis*-type larvae in experimental animals. *Canadian Journal of Zoology*, 41, 147–148.

Nascetti G., Paggi L., Orecchia P., Mattiucci S., Bullini L. 1983. Two sibling species within *Anisakis simplex* (Ascaridida: Anisakidae). *Parassitologia*, 25, 306.

Nascetti G., Paggi L., Orecchia P., Smith J. W., Mattiucci S., Bullini L. 1986. Electrophoretic studies on the *Anisakis simplex* complex (Ascaridida: Anisakidae) from the Mediterranean and North-East Atlantic. *International Journal for Parasitology*, 16, 633–640.

Oishi K., Oka S., Josho S. 1969. An introduction to food hygiene of the *Anisakis* larva. Hakodate Food Science Research Society (Pamphlet), Hokkaido, Japan (in Japanese).

Orecchia P., Paggi L., Nascetti G., Bullini L., Mattiucci S. 1983. Genetic differentiation between *Anisakis simplex* A, *A. simplex* B and *A. physeteris* (Ascaridida: Anisakidae). *Parassitologia*, 25, 311.

Oshima T. 1972. *Anisakis* and anisakiasis in Japan and adjacent area. In: *Progress of medical parasitology in Japan*. 4. (Eds. K. Morishita et al.). Meguro Parasitological Museum, Tokyo, 301–393.

Otsuru M., Hatsukano T., Oyanagi T., Kenmotsu M. 1965. The visceral migrans of gastro-intestinal tract and its vicinity caused by some larval nematode. *Japanese Journal of Parasitology*, 14, 542–555 (in Japanese).

Oyanagi T. 1967. Experimental studies on the visceral migrans of gastro-intestinal walls due to *Anisakis* larvae. *Japanese Journal of Parasitology*, 16, 470–493 (in Japanese).

Paggi L., Orecchia P., Bullini L., Nascetti G., Mattiucci S. 1983. Electrophoretic identification of *Anisakis* larvae from Mediterranean and North Atlantic (Ascaridida: Anisakidae). *Parassitologia*, 25, 315.

Petithory J. C., Pangam B., Buyet-Rousset P., Pangam A. 1990. *Anisakis simplex*, a co-factor of gasric cancer? *Lancet*, 1002.

Rokicki J., Berland B., Wróblewski J. 1997. Helminths of the harbour porpoise, *Phocoena phocoena* L., in the southern Baltic. *Acta Parasitologica*, 42, 36–39.

Skóra K., Kuklik I., Rokicki J. White-beaked dolphins *Lagenorhynchus albirostris* in the Polish zone of the Baltic Sea. *Aquatic Mammals*, (in press).

Smith J. W. 1989. Ulcers associated with larval *Anisakis simplex* B (Nematoda: Ascaridoidea) in the forestomach of harbour porpoises *Phocoena phocoena* (L.). *Canadian Journal of Zoology*, 67, 2270–2276.

Tanabe M., Miyahira Y., Okuzawa E., Segawa M., Takeuchi T., Shinbo T. 1990. A case report of ectopic anisakiasis. *Japanese Journal of Parasitology*, 39(40), 397–399.

Tanaka H., Takata S., Nishimura T., Watanabe S. 1968. A case report of *Anisakis* larva penetration in the pharynx mucosa. *Japanese Journal of Parasitology*, 17, 641 (in Japanese).

Usui M., Ashizawa H., Nosaka D., Tateyama S. 1973. Studies on swine gastric anisakiasis. 1. Morphological findings on worms. *Bulletin of the Faculty of Agriculture. Miyazaki University*, 20, 169–177 (in Japanese).

Van Thiel P. H., Van Houten H. 1967. The localization of the herring-worm *Anisakis marina* in-and outside the human gastro-intestinal wall (with a description of the characteristics

of its larval and juvenile stages). *Tropical and Geographical Medicine, Amsterdam,* 19, 56–62.

Van Thiel P. H., Kuipers F. C., Roskam T. H. 1960. A nematode parasitic to herring, causing acute abdominal syndromes in man. *Tropical and Geographical Medicine, Amsterdam,* 12, 97–113.

Yokogawa M., Yoshimura H. 1967. Clinicopathologic studies on larval anisakiasis in Japan. *American Journal of Tropical Medicine and Hygiene,* 16, 723–728.

Young P. C., Lowe D. 1969. Larval nematodes from fish of the subfamily Anisakinae and gastro-intestinal lesions in mammals. *Journal of Comparative Pathology,* 79, 301–313.