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Few studies describe the parasites of pigs bred in the wild state, although pigs are a known reservoir of trematode infestation. This article reports the results of a retrospective study carried out from January 2003 to June 2007 on 3021 Nebrodi Black male and female pigs, regularly slaughtered, aged between 8 months and 4 years. *Fasciola hepatica* and *Dicrocoelium dendriticum* flukes were detected in 143 (4.37%) of 3021 livers. The predominant histological features were multifocal to diffuse chronic hepatitis, with fibrosis and severely thickened walls of the bile ducts and chronic parietal, sometimes nodular inflammation. *F. hepatica* infestation was frequently associated with marked hyperplasia and hypertrophy of the submucosal glands. The study results confirm the important role swine play in the transmission of trematode infestations, indicate the prevalence of these parasites in the Nebrodi Park area, and draw attention to the need for a prophylaxis plan to prevent the spread of infestation to ruminants and humans living in the area.

1. Introduction

In the last 50 years, intensive pig breeding has reduced the incidence of parasite infestation and the number of different parasite species. Because of the lack of spontaneous feeding on the natural external environment, helminth infestations in pigs housed in intensive systems have nearly disappeared. Nonetheless, infestations by parasites with a direct or an indirect biological cycle can often be observed in swine living in the wild, such as Nebrodi Black pigs (Poglayen et al., 1991).

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Here, we describe trematode infestations *(Fasciola hepatica* and *Dicrocoelium dendriticum)* and liver involvement in a wild population of Black pigs living in the Nebrodi Park protected area (Messina, Sicily).

Trematodes are flukes of the Trematoda class in the phylum Platyhelminthes that affect animals and humans. *F. hepatica* may be the main pathogen for fascioliasis, or ''liver rot'' quite common in ruminants, but rare in pigs, horses, dogs, cats, rabbits, guinea pigs, squirrels, deer and beavers (Jones et al., 1997). However, a wide variety of mammals including humans (Bergsmann et al., 1957; Gironi and Brambilla, 1964; Ziegler et al., 1975; Beresford, 1976; Jones etal., 1977; Helfensteinetal.,2000; Cristmann et al., 2002; Sorokina et al., 2003) may be infested by ingestion, with watercress ingestion the most common

cause of the infestation. The parasite is much less well adapted to non-ruminants hosts, and ectopic infestations may develop in the lungs or subcutaneous tissues.

These parasites have a worldwide distribution throughout the temperate and tropical regions.

The life cycle of *F. hepatica* needs an intermediate host, typically snails of the genus *Lymnaea.* Eggs hatch miracidia which penetrate the snails and undergo a series of developmental stages (sporocysts-redia-daughter redia), leading to the release of cercaria. The cercariae are released into the external environment and deposited on vegetation, where they remain until ingested by the final host.

D. dendriticum is another trematode responsible for hepatobile damage. The adult worms reside in the bile ducts of ruminants (Camara et al., 1996; Otranto and Traversa, 2002, 2003), and can also be found in camels, horses, pigs, rabbits, dogs, cervids but rarely in humans (Vasallo Matilla, 1971; Bygbjerg and Gomme, 1980; Azizova et al., 1988; Drabick et al., 1988; Helmy and Al Mathal, 2003; Rack et al., 2004). The adult worms produce eggs which are excreted with the feces. Two intermediate hosts are known: a terrestrial snail and an ant. The final host is infested by ingestion of ants during grazing.

All sorts of mammalian species are exposed to trematode infection, but species susceptibility and pathological findings differ widely. Ruminants (cattle and sheep) have low resistance to these parasites, especially to *F. hepatica* which produces severe bile duct lesions. Cattle are more resistant to fascioliasis than sheep, and acquired resistance is well known in the adult cow (Doyle, 1973; Haroun and Hillyer, 1986).

F. hepatica may occasionally parasitize other mammals, particularly pigs and horses (Nansen et al., 1975; Prestwood et al., 1975; Owen, 1977; Luengo et al., 1984; Apt et al., 1993). Pigs are considered highly resistant to this parasite, and in experimentally infested pigs an intense fibrotic tissue encapsulating the flukes has been reported (Nansen et al., 1972).

Here we describe the macroscopical and histologic features of lesions observed in cases of natural distoma-tosis in feral Black pigs caused by parasites of the Fasciolidae family (F. *hepatica)* and of the Dicrocoelidae family *(D. dendriticum).*

2. Materials and methods

The study was conducted over a 54-month period from January 2003 to June 2007 on 3021 Nebrodi Black pigs, regularly slaughtered at a private slaughterhouse under official veterinary control. The pigs were between 8 months and 4 years old, both male and female, and came from different farms in the Nebrodi Park area.

Livers samples were collected from October to March, in the period when most Nebrodi Black pigs are slaughtered for the production of typical local products.

All livers were macroscopically observed and those found positive for flukes were sent to the laboratory for detailed investigation. The gall bladders and common bile duct were opened and grossly visible flukes were identified. The parasites were collected and fixed in 10% buffered formalin. After washing under running water (12-24 h), the parasites were diaphanized in lactophenol of Amman and then examined by light microscopy (Leica Microsystems S.p.A., DM LS2) for identification.

Prevalence, mean intensity of infection, standard deviation, median and range were calculated for each detected parasite species.

Samples with gross appearance of evident lesions (n = 96) were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin. The composition of glandular secretions was analyzed by histochemical staining with periodic acid Schiff (PAS) and Alcian blue using the critical electrolyte concentration technique (CEC) at different concentrations of magnesium chloride (0.1-1.0 M) (Pearse, 1985; Salvetti et al., 2003).

Small samples from six livers were also processed for electron microscopy. After formalin fixation, the tissue was post-fixed in 1% osmium for 2 h, dehydrated in acetone and embedded in Spurr resin. Semithin sections were stained with toluidine blue and ultrathin sections were contrasted with uranyl acetate and lead citrate. The grids were read on a transmission electron microscope (Zeiss mod. EM 109 **JD).**

3. Results

F. hepatica and *D. dendriticum* flukes were detected in 143 (4.73%) of 3021 livers: 54 livers contained *F. hepatica* and 64 contained *D. dendriticum* flukes; co-infestation with both parasites was determined in 25 pigs aged >12 months (Table 1).

The intensity of infestation varied across the study period, since most samples were collected from October to March of each year (Table 2). The mean intensity of infestation, standard deviation, median values and range are shown in Table 1. Except for the 14 cases of massive infestation (10-50 flukes in the liver sample), in the remaining 129 samples, 1-10 flukes per sample were detected (Table 1). Almost all (90%) affected pigs were aged between 10 months and 2 years and the most intense infestation was detected in animals aged >12 months.

Table 1
Number of flukes recovered, mean, standard deviation, median values and range.

Parasite species	No. of positive livers (%)	No. of flukes recovered (no. of cases)	Mean	S.D.	Median values	Range
Fasciola hepatica	54 (37.76)	1–10 (48)	5.16	2.75	5	1–10
		10–50 (6)	13.5	2.73	13	11–18
Dicrocoelium dendriticum	64 (44.76)	1–10 (56)	7.19	1.99	7.5	3–10
		10–50 (8)	23.25	5.23	23	15–31
Co-infestation	25 (17.48)	*Fasciola hepatica* 1–10	4.04	2.16	4	1–8
		Dicrocoelium dendriticum 1–10	8.36	1.68	9	5–10

Table 2
Number of Nebrodi Black pigs collected in different periods between 2003 and 2007 and prevalence of liver flukes.

Year	Period	No. of pigs	No. of parasitized livers	%
2003	January 2003–March 2003	314	17	5.14
	April 2003–September 2003	17		
2004	October 2003–March 2004	717	26	3.44
	April 2004–September 2004	38		
2005	October 2004–March 2005	407	8	1.86
	April 2005–September 2005	24		
2006	October 2005–March 2006	798	37	4.40
	April 2006–September 2006	42		
2007	October 2006–March 2007	647	55	8.28
	April 2007–June 2007	17		
		Total 3021	Total 143 (4.73%)	Mean 4.62

Histological examination was carried out on 96 livers that showed gross lesions: 47 showed lesions caused by *F. hepatica* and 49 showed lesions caused by *D. dendriticum.*

The livers infested by *D. dendriticum* showed severe lesions associated with many different parasites of the bile ducts (Figs. 1 and 2). Multifocal areas of fibrosis with granulomatous foci characterized by mixed cells infiltration and numerous eosinophils were present. In one liver sample the lesions caused by *D. dendriticum* were especially conspicuous, with severe diffuse sclerosis and severe suppurative angiolitis involving the entire organ. Also observed were multifocal granulomas with many parasite eggs surrounded by mononuclear inflammatory cells and giant cells (Fig. 3). Dilated bile ducts with normal wall thickness were noted. No calcium deposits were observed.

Infestation by *F. hepatica* involving the entire organ was determined in 47 livers. Macroscopic migratory tracts were diffuse on the surface. The gall bladder and bile ducts appeared dilated with thickened walls and the inner surface was covered by a yellow-greenish material. Numerous mature flukes were observed in the bile ducts (Fig. 4).

Histologically, a perilobular chronic hepatitis with pronounced increase of interlobular connective tissue was seen. The biliary ducts showed thickened walls with parietal chronic inflammation involving the mucosa and submucosa. The inflammatory cells (lymphocytes, monocytes, plasma cells, macrophages and numerous eosinophils) were organized in granulomatous nodules around

Fig. 1. Pig, liver. Surface disseminated with evident tracks of migratory flukes in *Dicrocoelium dendriticum* infestation.

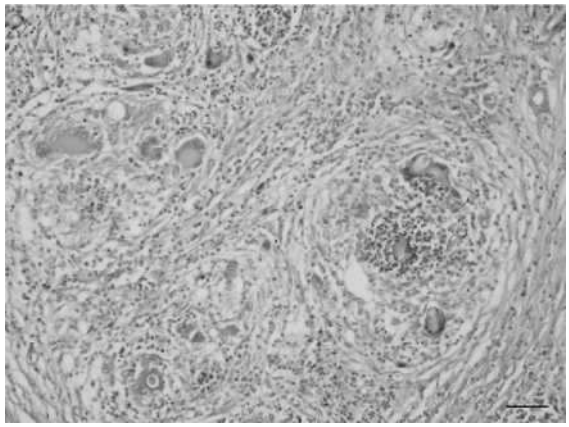

Fig. 2. Pig, liver. Bile duct containing a cross-section of a *Dicrocoelium dendriticum* fluke. Severe inflammatory and reparative reaction present in the surrounding parenchyma. Hematoxylin and eosin. Bar = 250 mm.

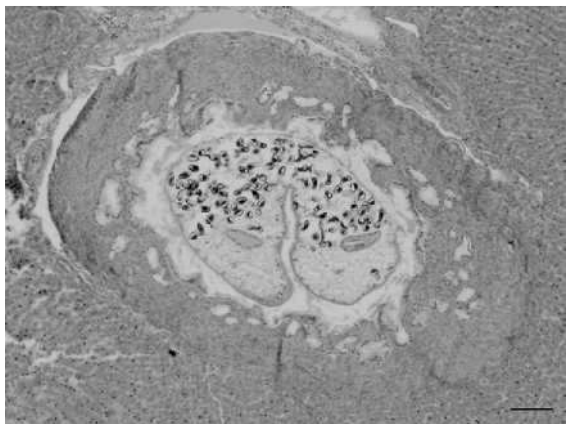

Fig. 3. Pig, liver. Granuloma with numerous parasite eggs, surrounded by mononuclear inflammatory cells and giant cells. Hematoxylin and eosin. Bar= 100 mm.

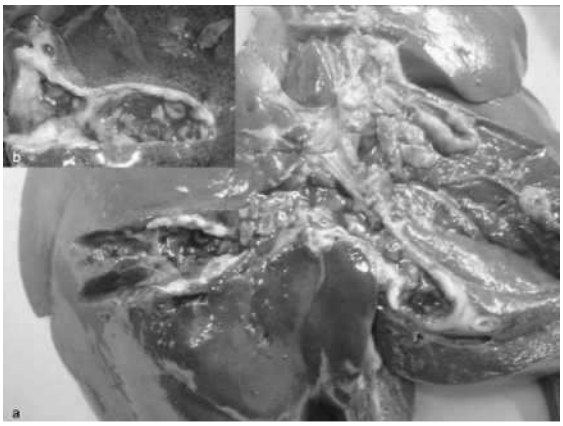

Fig. 4. Pig, liver surface. (a) Gall bladder and dilated bile ducts with thickened walls and an inner surface covered by a yellow-greenish material. Numerous mature *Fasciola hepatica* flukes in the bile ducts. (b) Gall bladder with evident thickened wall and chronic granulomatous inflammation.

the bile ducts. The surrounding parenchyma were infiltrated by a large number of cells, mainly eosinophils and lymphoid cells. Diffuse fibroblastic reaction noted throughout the liver was organized in small nodules associated with the inflammatory cells in some cases. The bile duct epithelium was frequently necrotic as was the submucosal layer (Fig. 5). In the major bile ducts, marked hyperplasia and hypertrophy of the branching adenomeres were observed in the submucosa. The proliferation composed of voluminous mucous cells with a spherical nucleus and clear cytoplasm was surrounded by fibrous tissue and infiltrated by lymphocytes and eosinophils. The glandular secretions stained strongly positive with PAS. The Alcian blue CEC method showed alcianophilia only at a concentration of 0.1 M magnesium chloride. Ultrastruc-tural investigations revealed the presence of glandular cells in the submucosa of the bile ducts. The cytoplasm of

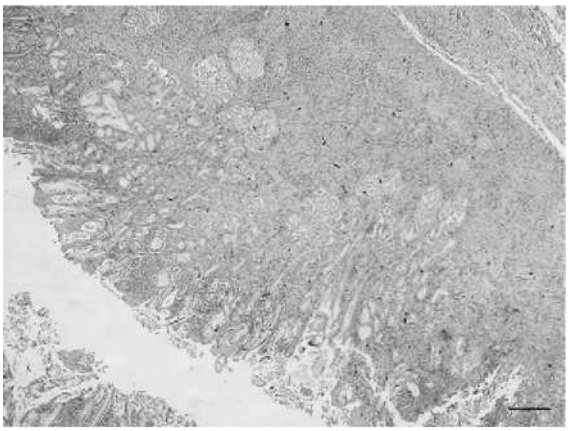

Fig. 5. Pig, liver. Bile ducts with parietal chronic inflammation involving the mucosa and the submucosa. Inflammatory cells (lymphocytes, monocytes, plasma cells, macrophages and numerous eosinophils) around the bile ducts and infiltrating the surrounding parenchyma. Marked hyperplasia and hypertrophy of submucosal glandular cells. Hematoxylin and eosin. Bar = 500 mm.

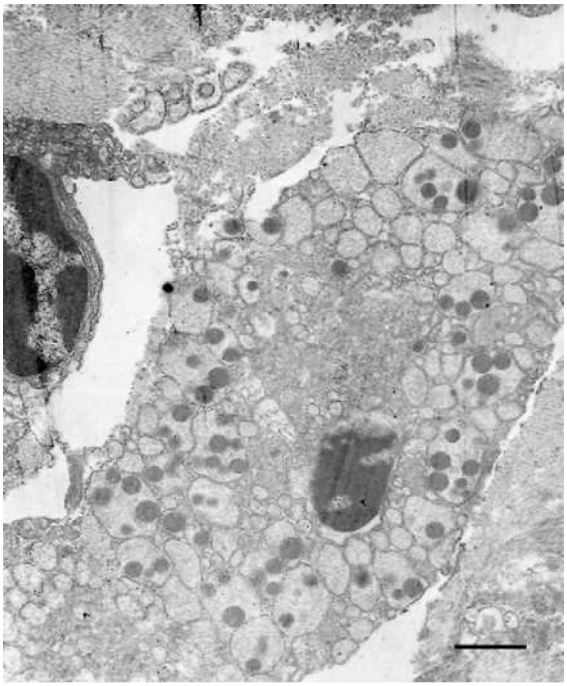

Fig. 6. Pig, liver. Glandular cell of the bile duct submucosa; the cytoplasm contains many clear vacuoles with dark granules. Electron microscopy. Bar = 1196 nm.

the cells was characterized by many clear vacuoles, with some dark granules that appeared to be prosecretory structures migrating to the apical cell surface and discharging their contents into the gland lumen (exocy-tosis) or to be directly released into the lumen (apocrine secretory mechanism) (Fig. 6).

Bile duct thickening and marked hyperplasia of the glands observed in the submucosa were detected only in the livers affected by fascioliasis and especially where infestation was massive (six cases).

4. Discussion and conclusions

Few published data describe the parasites of pigs bred in a wild state and of wild boars (Boch and Horchner, 1961; Foreyt and Tood, 1972; Prestwood et al., 1975; Cotteleer and Fameree, 1982; Arru et al., 1983; Barutzki et al., 1990; Poglayen et al., 1991; Eslami and Farsad-Hamdi, 1992; Bollo et al., 1993; Boucher et al., 2005; Jarvis et al., 2007). With this study, we were able to examine numerous feral pigs and so obtain interesting information on hepatic parasitosis in these animals. Of particular interest was finding parasites like *F. hepatica* and *D. dendriticum* which are considered infrequent in the pig. In a recent study by Valero and Mas-Coma (2000) on the viability of meta-cercariae of *F. hepatica* experimentally obtained from eggs shed by naturally infected sheep, cattle, pigs and donkeys on the Northern Bolivian Altiplano, it was found that the metacercarial viability in pigs is similar to that in sheep and cattle, suggesting that the pig may have a high transmission capacity. However, the descriptions of natural infestation by trematodes in this species are

sporadic (Foreyt and Tood, 1972; Barutzki et al., 1990; Eslami and Farsad-Hamdi, 1992; Apt et al., 1993; Bollo et al., 1993; Valero and Mas-Coma, 2000) and detailed investigations about lesions are rare (Ross et al., 1967; Dalchow et al., 1971). Experimentally, the pig is a resistant animal to these parasites. Trematode infestation causes serious hepatic lesions mainly characterized by severe fibrosis (Horchner and Dalchow, 1972; Nansen et al., 1972, 1974).

In the feral Black pigs examined in our study, the lesions caused by *F. hepatica* were noted to be severe and diffuse. In cattle, this parasite generally involves the visceral aspect of liver, causing serious chronic angiocolitis with secondary increased bile duct wall thickness and calcium deposition. In sheep and other species, the lesions are more widespread, calcium deposition is normally absent, and fibrous reaction is less severe, allowing to the parasite to survive and dilate the bile duct lumen. In pigs and horses, the lesions are diffuse, calcification is absent, and the duct walls are rarely thickened with severe glandular proliferations. In our study, the bile duct walls were conspicuously thickened and severe hypertrophy/hyper-plasia of the mucous glands was detected in almost all cases. Generally, the bile ducts are composed of a simple cuboidal/columnar epithelium, with goblet cells in the largest ducts, and mucous and serous glands disseminated in the mucosal layer. In the pig, however, this glandular tissue is normally scarce (Nickel et al., 1979). The conspicuous engrossment of these glands represents a reaction to pathological insults caused in this case by the passage of *Fasciolae* which are able to survive at length in the pig's bile ducts.

In experimental studies on pigs, glandular hyperplasia has been observed in association with marked hyperplasia and hypertrophy of the epithelium of the bile ducts (Nansen et al., 1972). In our study, we never observed nodular lesions like those in domestic pigs Nieberle and Cohrs (1970) reported to be caused by larval forms during migration in the liver parenchyma.

Histochemical staining to reveal the nature of the glandular secretions indicated that they contain both neutral (PAS positive) and acidic non-sulphated mucosub-stances. In fact, the Alcian blue CEC method stained the secretions blue only at a very low electrolyte concentration (0.1 M); this excluded sulphated glycosaminoglycans, but neutral glycoproteins were revealed by PAS staining.

Parasitism is a biological indicator of an animal's welfare and state of health. Severe natural infestation by trematodes in feral Black pigs reflects the prevalence of these parasites in the Nebrodi Park area. The Nebrodi Park is a natural park near the city of Messina. It encompasses some of Sicily's most extensive woodlands, along with meadows and cultivated lands. Because of the park's environmental variety, it sustains complex faunal communities of small mammals, reptiles, amphibians and nesting or migratory birds. Many different animal species such as cattle, small ruminants, horses and pigs breed within the park territory.

The study by Valero and Mas-Coma (2000) confirmed the important role of swine in the transmission of fascioliasis and the great importance for controlling human and animal fascioliasis. The presence of many animal species breeding in the same habitat, such as pigs and cattle in the Nebrodi, requires mandatory activation of intense prophylactic measures to prevent the spread of this parasitosis and of other infectious diseases (such as tuberculosis) to ruminants and humans.

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