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Source: Journal of Wildlife Diseases, 14(3) : 371-377

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-14.3.371

CHOLANGITIS ASSOCIATED WITH SPECIES OF *Progamotaenia* (CESTODA: ANOPLOCEPHALIDAE) IN THE BILE DUCTS OF MARSUPIALS

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Abstract: Gross and histopathologic changes due to infection with *Progamotaenia festiva* and *P. effigia* in the bile ducts of the marsupials, *Macropus rufus, M. giganteus, M. fuliginosus, Lagorchestes conspicillatus, Vombatus ursinus* and *Lasiorhinus latifrons* are described. The integrity of the bile duct mucosa was not impaired but varying degrees of hyperplasia and hypertrophy of mucosa and mucosal glands, inflammatory infiltration and fibrosis were found. Portal fibrosis was the prominent reaction in *Lasiorhinus latifrons*. Reduced prevalence of cestode infection was recorded in two populations of *M. giganteus* in which *Fasciola hepatica* also was recovered.

INTRODUCTION

Several species of anoplocephalid cestode occur in the bile ducts of mammals, namely *Stilesia hepatica, Thysanosoma actinioides* and *Wyominia tetoni* in ruminants, *Anoplocephaloides floresbarroetae* in the rabbit, *Sylvilagus brasiliensis,* and three species of the genus *Progamotaenia* in the Australian marsupials. The species in marsupials are *Progamotaenia festiva,* a widely distributed species occurring in macropods and wombats, *P. effigia,* a cestode restricted to one species of kangaroo, *Macropus fuliginosus,* and *P. diaphana* restricted to wombats.[2]

The pathology associated with *S. hepatica, T. actinioides* and *W. tetoni* infection has been described,[1,5,7] but there is no information in the literature on the effects of *Progamotaenia* spp. on the biliary systems of their hosts.

It is the purpose of this communication to describe the comparative pathologic features of *Progamotaenia* spp. infection in 6 marsupial hosts.

MATERIALS AND METHODS

Kangaroos and wombats were collected by shooting, mainly at night with the aid of a spotlight. As soon as possible after the death of the animal, pieces of liver, bile ducts or gallbladder were fixed in 10% formol saline or Bouin's fluid with cestodes *in situ.* Other cestodes were removed from the bile ducts, relaxed in water, fixed in 10% formalin or 70% ethanol, and were stained, mounted and identified. Specimens were also collected from noninfected kangaroos for use as controls. Livers were examined from 93 red kangaroos, *Macropus rufus,* 99 eastern grey kangaroos, *Macropus giganteus,* 39 western grey kangaroos *Macropus fuliginosus,* 15 common wombats, *Vombatus ursinus* and 5 hairy-nosed wombats, *Lasiorhinus latifrons.* Fixed liver samples were examined from approximately one quarter of the kangaroos collected.

In addition, three common wombats were collected as road kills, and therefore postmortem examinations were conducted several hours after death. Tissues were fixed in 10% formol saline.

Two fixed specimens of the spectacled hare-wallaby, *Lagorchestes conspicillatus,* were provided by Dr. J. Nelson, Monash University, Melbourne. The two specimens had been shot and the carcasses immediately perfused via the

left ventricle with 20% formol saline, followed by immersion of the animal in 10% formol saline. Both wallabies had cestodes in their bile ducts.

Tissues were embedded in paraffin and 6 μm sections cut from them were stained routinely with hematoxylin and eosin (H & E) or occasionally with Periodic acid Schiff (PAS) to evaluate mucus production and secretion.

RESULTS

Cestodes

P. festiva was found in 75 of 97 (77%) *M. giganteus*, 66 of 93 (71%) *M. rufus*, 2 of 2 (100%) *L. conspicillatus*, 5 of 5 (100%) *L. latifrons* and 2 of 18 (11%) *V. ursinus*. *P. effigia* was found in 23 of 39 (59%) *M. fuliginosus*. No *P. diaphana* was found.

Specimens of *P. festiva* collected from *M. giganteus* and *M. rufus* were between 19 and 34 cm long, as reported earlier[2] for this species. Infection was common in both host species and up to 20 tapeworms were extracted from individual animals. Specimens from *V. ursinus* were shorter (<10 cm) but were as wide as the larger specimens from macropods, the internal morphology not differing from earlier descriptions.[2] The worms were gravid but only 1-2 were present in each of 2 infected hosts. Specimens from *L. latifrons* were considerably smaller (5-7 by 0.3-0.5 cm), were quite diaphanous when fresh and the scolex of several worms contained black pigment. Testes were distributed either in a single band proximal to the uteri or in two lateral groups on either side of the proglottis medulla. Cestodes were numerous in each animal examined.

Gross Pathologic Findings

In macropods infected with cestodes, the main hepatic ducts were prominent, dilated, and had slightly thickened walls. Cestodes were also found in the gallbladder and cystic duct. In *V. ursinus*, the principal hepatic duct walls were thickened and more prominent than usual. Cestodes were found throughout the biliary system in *L. latifrons* and even small portal triads were very prominent on the cut surface, appearing as white foci up to 4 mm in diameter. Major bile ducts were also thickened.

Histopathologic Features

Based on comparisons made with tissues examined from non-parasitized hosts, the changes associated with *P. festiva* infection in 5 marsupials are summarized (Table 1).

Red kangaroos

In *M. rufus* with *P. festiva* infection, lumina of main bile ducts were dilated and often full of cestodes (Fig. 1). The epithelium was intact with no evidence of erosion and epithelial cells were hypertrophic. The mucosa was slightly thickened and occasionally folded. There was mild proliferation of mucous glands and increased mucus production. Fibrous thickening of the duct wall was slight and only a light mononuclear cell and eosinophil infiltration was seen. In larger portal triads, hypertrophic changes in the epithelium were mild and there was only mild fibrosis. No changes were seen in smaller triads or the liver parenchyma. Of the various hosts examined, the biliary system of *M. rufus* was the least altered by the presence of cestodes.

Eastern grey kangaroos

In *M. giganteus* with *P. festiva*, the mucosa of the main bile ducts was much thicker, the hyperplastic changes more pronounced with greater folding, and mucous glands had dilated lumina and excess secretion. There was mild fibrous thickening of the wall and a moderate, diffuse cellular infiltration of the duct wall and the lamina propria of the mucosa. Mild hyperplastic changes in bile duct epithelium were present in medium-sized portal triads.

TABLE 1. Features of the cholangitis associated with *Progamotaenia festiva* infection in 5 marsupial hosts: Comparison by lesion score.

	Macropus rufus	*Macropus giganteus*	*Lagorchestes conspicillatus*	*Vombatus ursinus*	*lasiorhinus latifrons*
Major Bile Ducts					
mucosa thickened	+	++	-	+	-
epithelium, hypertrophic	++	++	-	+	+
epithelium, hyperplastic	+	++	-	+	+
mucous glands, proliferation	++	+++	+	++	-
mucous glands, secretion	++	++	++	++	-
duct wall, fibrosis	+	++	+	+++	++++
duct wall, infiltration	+	+++	-	-	-
Larger Portal Triads					
epithelium, hypertrophic	+	+	++	+	+
epithelium, hyperplastic	-	+	-	-	-
portal fibrosis	+	++	++	++	++++
bile duct proliferation	+	+	++	+	++
Small Portal Triads					
bile duct proliferation	-	-	+	-	-
portal fibrosis	-	-	+	-	+

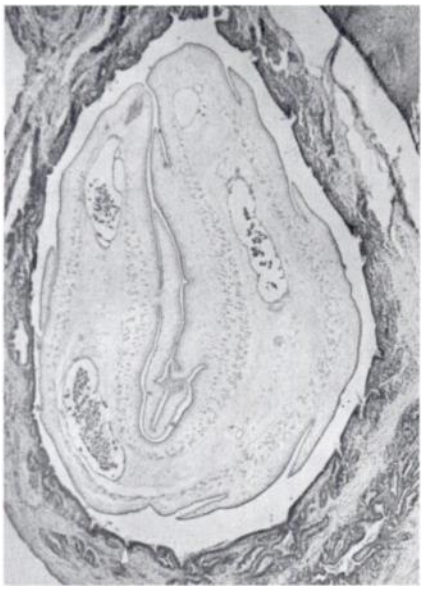

FIGURE 1. *Progamotaenia festiva* in the main bile duct of *Macropus rufus*. Bile duct eipthelium is intact, the mucosa only slightly increased in height, with mild fibrous thickening of the wall. H & E. × 38.

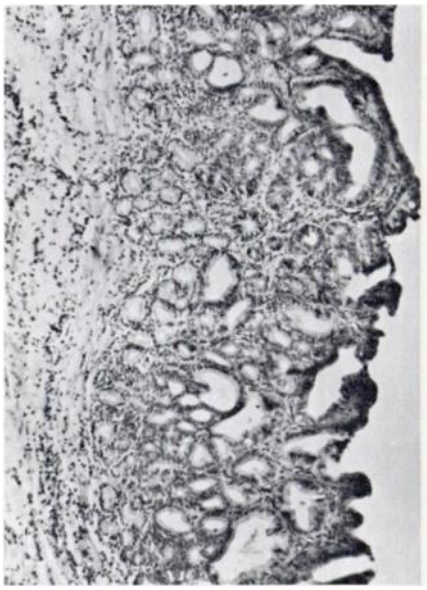

FIGURE 2. *Progamotaenia effigia* infection in *Macropus fuliginosus*. Mild hyperplasia of main bile duct epithelium, with eosinophil and mononuclear cell infiltration. There is proliferation of mucous glands as well. H & E. × 200.

Western grey kangaroos

The bile duct changes seen in *M. fuliginosus* infected with *P. effigia* (Fig. 2) were most similar to those seen in *M. giganteus* with *P. festiva* infection. There was greater cellular infiltration in the mucosa and wall of the main bile duct and greater proliferation of mucous glands. Fibrosis was also a more prominent change, and was seen in large portal triads.

Spectacled hare-wallabies

In *L. conspicillatus*, epithelial hypertrophy and dilatation of lumina of mucous glands with excessive mucus secretion were the major changes associated with *P. festiva* infection. The bile duct epithelium was intact, hypertrophic in larger portal triads, and there was no evidence of inflammatory cell infiltration. Portal fibrosis was evident in both large and smaller triads and there was a mild proliferation of bile ductules in many of the smaller triads.

Common wombats

In *V. ursinus* with *P. festiva* infection, only the main bile ducts were affected. The epithelium was intact and epithelial cells were hypertrophic. Hypertrophy and proliferation of mucous glands was evident, with greatly increased mucus secretion (Fig. 3). There was no cellular infiltration but the bile duct wall was considerably thickened by a band of fibrous tissue.

Hairy-nosed wombats

In *L. latifrons* infected with *P. festiva*, the epithelium of major bile ducts was intact but mildly hypertrophic and

hyperplastic. There was no cellular infiltration around bile ducts nor was there any hypertrophy of mucous glands. All large and smaller portal triads showed very marked fibrosis and a mild proliferation of bile ducts (Fig. 4).

DISCUSSION

P. festiva provoked very mild hypertrophic and hyperplastic changes in the bile duct mucosa of *M. rufus*. More extensive hypertrophic and hyperplastic changes and inflammation occurred in infected *M. giganteus* and a similar reaction was found in *M. fuliginosus* infected with *P. effigia*. Excess mucus production and mild fibrosis characterized the major changes in *L. conspicillatus*. In both *V. ursinus* and *L. latifrons* infected with *P. festiva*, fibrosis of bile duct walls was the major feature of the lesion, particularly in the latter host.

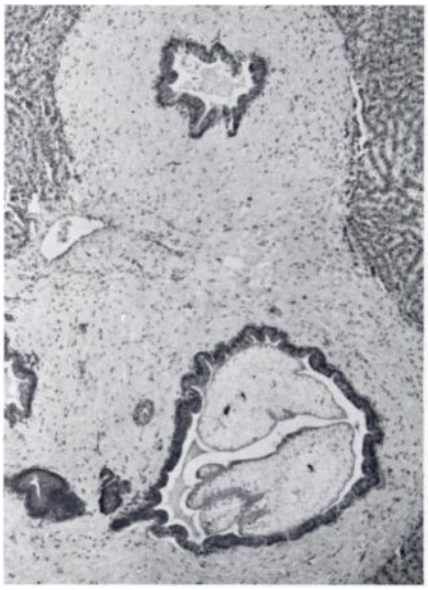

FIGURE 4. Marked fibrosis of a large portal triad in response to *P. festiva* infection in a dilated bile duct of *Lasiorhinus latifrons*. Bile duct epithelium is intact and cells are hypertrophic. H & E. × 50.

FIGURE 3. Fibrous thickening of a major bile duct in *Vombatus ursinus* infected with *P. festiva*. Hyperplasia and hypersecretion of mucous glands is evident. PAS. × 50.

Fibrosis, bile duct proliferation, hyperplasia and cellular infiltration have been described in sheep infected with *T. actinioides*,[1] and *S. hepatica* in impala.[7] However, in the latter case, the photomicrograph presented has sections through *Cooperioides hepatica* labelled as *S. hepatica*.[7] *W. tetoni* has also been reported as causing biliary hyperplasia and fibroplasia, occasionally occluding bile ducts and causing a chronic icteric condition in bighorn sheep *(Ovis canadensis)*.[5]

Lesions caused by *Progamotaenia* spp. are therefore similar to those caused by other cestode species, though the variety of reactions in different hosts such as described above has not previously been

reported. The lesions in all species of marsupial hosts were mild and consisted essentially of changes related to increased mucus secretion due to the presence of the parasite.

The lesions are particularly mild when compared with other biliary parasites such as *Fasciola hepatica* which actively erode the bile duct epithelium. The latter parasite induces very marked hyperplasia and inflammation of the biliary mucosa both in *M. giganteus* and *V. ursinus* (our unpublished observations).

A reduced prevalence of *P. festiva* infection in *M. giganteus* was found in areas where *F. hepatica* was also recovered. In the Grampian mountains of southwestern Victoria, 3 populations of *M. giganteus* were examined. *P. festiva* infection was found in 100% of kangaroos sampled on the hillsides east (19 of 19) and west (7 of 7) of the Victoria Valley. However, on the valley floor (near Mirranatwa) where 10 of 19 (53%) *M. giganteus* were found infected with *F. hepatica,* only 10 of 19 (53%) harbored *P. festiva.* Of these kangaroos, only 3 (16%) were infected with both helminths and *F. hepatica* burdens were very light (1-5 flukes). Similarly, in a reserve near Canberra, A.C.T. where 12 of 14 (86%) *M. giganteus* harbored *F. hepatica, T. festiva* was found in only 1 animal (7%). In the kangaroo with concurrent infections, 5 *F. hepatica* were recovered and only 2 of these were recently gravid. This apparent deleterious effect of *F. hepatica* infection on a cestode inhabiting the bile duct is similar to findings reported in mice experimentally infected with *F. hepatica* and *Hymenolepis microstoma.*[4,6]

Attempts to correlate the degree of pathologic reaction with the suitability of the host were not successful. *P. festiva* occurs very commonly and in large numbers both in *M. rufus* and *M. giganteus* in southeastern Australia (our unpublished observations) yet obvious differences exist in the reaction of the two hosts to the parasite.

P. effigia is found only in *M. fuliginosus,* and this must be considered the normal host for the parasite, even though a greater reaction occurs to this parasite than to *P. festiva* in *M. rufus.*

P. festiva is a common parasite of various macropods but occurs infrequently in *Vombatus ursinus.*[2] The specimens reported here confirm earlier observations[2] on morphology except that all specimens were gravid. The specimens were shorter than those found in the large macropodid hosts.

P. festiva is reported from *L. latifrons* for the first time. *P. diaphana* has been recorded[2] from this host but the specimens were re-examined and compared with new, more extensive collections and found to be *P. festiva.* Specimens from *L. latifrons* differ from more typical specimens in being shorter and narrower, almost transparent when fresh, in having slightly fewer testes per proglottis and in having black pigment in the scolex. None of these features is sufficiently reliable to distinguish them from *P. festiva.* They are distinguished from *P. diaphana* only in lacking vaginal atrophy following insemination and differences in the morphology of the pyriform apparatus. The specimens reported here, together with those reported earlier[2] as *P. diaphana* from *L. latifrons* are tentatively identified as *P. festiva. P. diaphana* is therefore known only from the type specimens of uncertain host origin *(Phascolomys wombat)* and remains to be identified in free-living wombats. *P. diaphana* with black pigment in the scolex has been reported[3] from a "Tasmanian wombat", *V. ursinus.* Communications with Dr. Supperer by a colleague, Dr. I. K. Barker, have established that their specimens came from *L. latifrons* (I. K. Barker, pers. comm.), not *V. ursinus.* They are remarkably similar to specimens described above.

Acknowledgements

We wish to thank Mr. M. Gaughwin for access to specimens of *L. latifrons*. Part of the work was supported by a grant from the Australian National Parks and Wildlife Service.

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Received for publication 21 December 1977