COMPARATIVE AND GEOGRAPHIC PATHOLOGY OF OPISTHORCHIS VIVERRINI INFECTION: NATURALLY INFECTED CATS

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The liver fluke Opisthorchis viverrini is a medical and parasitological problem of considerable magnitude in the human population of northeast Thailand. (First slide - Map of Southeast Asia). The shaded area, the Korat Plateau, is populated predominantly by a Thai-Lao ethnic group whose dietary habits include eating raw fish. Wykoff has estimated that over 3 1/2 million people are infected in Thailand and that several millions more are likely to be infected in nearby Laos, Vietnam and China. Over 5,000 persons were examined by means of a single stool specimen concentrated by the formalin-ether technique. In all, 78% were found to harbor the parasite, and 90% of all those examined who were over the age of ten were infected. Approximately 40% of some 80 dogs and 60% of some 55 cats were positive.

(Slide 2 - Life Cycle). The adult fluke develops in the liver of man, cats or dogs and ova are passed through the bilirary system into the stool. A ciliated larvae, the miracidium, hatches from the eggs after being eaten by snails of the genus Bithynia and a sporocyst develops within the snail. Redia develop within the sporocyst and produce cercariae which escape from the snail and penetrate into fresh water fish. Puntius orphoides, Hampula dispar, and Cyclocheilichthys siaja are the most frequently infected species. The cercariae lose their tails after penetrating into the muscles. They encyst and become "metacercariae". When raw or insufficiently cooked fish are eaten by a definitive host, the metacercariae excyst in the gastrointestinal tract and migrate up the bile ducts into the liver. The flukes begin to produce eggs in 30 to 40 days.

Sadun, in 1955, reported that the people of the area associated a syndrome with the presence of this parasite. He found that the earliest indications of illness were reported to be a generalized feeling of malaise, abdominal discomfort and occasional diarrhea, sometimes followed by transient urticaria, jaundice and pain at the site of the liver. In one village, he found that almost 17% of the individuals he examined had enlarged livers, however, the percentage of enlarged livers in the group with Opisthorchis ova in the stool was only slightly greater than in thos individuals without ova. The pathologic changes caused by Opisthorchis viverrini have not been described in detail; however, Promas in 1926 described marked thickening of the bile ducts and gall bladder in a 17 year old boy.

The objective of this study was to describe the lesions caused by Opisthorchis viverrini in the natural host before a study of experimentally produced infections in cats, rabbits and monkeys was begun.

Twenty five domestic cats were obtained from rural villages and examined for ova. Sixty eight per cent were positive. From stool examinations on three consecutive days, the number of eggs being passed was determined. Five animals with known egg counts were then sacrificed and the number of flukes in the livers were counted. By this means it was roughly estimated that an adult worm passes 2,000 eggs per day. Eleven positive cats were then completely autopsied.

(Slide 3 - Table I). Table I shows the estimated number of flukes in the autopsied cats, to nearest 10, except for those estimated to be carrying less than 10. For all eleven animals the average number of worms is 40. Except in animals 10 and 11, the micorscopic impression of the number of flukes and the estimates of flukes based on egg counts agree fairly well. If it is valid to say that the age of these cats correspond roughly to the length and weight of the animal, it might be assumed that more mature flukes are present, and they may therefore be producing more eggs per worm. However, so far I have been unable to make any judgement about the maturity of flukes in histologic sections. In addition, it should be noted that in these last 2 cats, the degree of scarring around ducts was significantly greater than in other animals. In the 2 cats with very few worms (1 and 6) there was very little scarring around the ducts.

On examination of the fresh livers, flukes could be expressed easily from the common bile duct and from cut surfaces of the liver. Some livers from large cats which appeared to have carried heavy infections for a long time had scarred surfaces, especially at the periphery of the lobes. The cut surfaces of the livers with infections of sufficient duration showed ectatic bile ducts with thickened walls. Occasionally, a few flukes were present in the gall bladder. No biliary calcali were found.

The microscopic changes vary from the slight scarring and mild inflammation seen in the two animals with only a few flukes to an intense inflammatory reaction in some cases, and severe scarring in others.

(Slide 4). This photomicrograph from the cat estimated to have only 4 flukes shows a nearly normal liver; however, there is a slight increase in connective tissue around the duct. (Slide 5). This shows the one fluke found microscopically. It is distending the duct and there is a chronic inflammatory reaction in the wall.

(Slide 6). In some more heavily infected livers, there is purulent exudate in the large bile ducts, but in some it is impossible to find any exudate. Here is a large thick-walled ectatic duct with many crypt-like spaces in the wall, purulent exudate is present around the fluke and there is an infiltration of the wall by neutrophiles and eosinophiles. (Slide 8). Very small portal areas large enough

to hold a fluke, are severely scarred and acutely inflammed. In this duct the epithelium has broken down. Where both pus and flukes are present in ducts, breakdown of the epithelium is occasionally seen but more often the epithelium is intact. In this section it appears that only a few epithelial cells have been destroyed. In most animals, mitotic figures are not difficult to find in the duct epithelium. (Slide 9).

In some livers, although flukes are abundant, there is little inflammatory reaction. (Slide 10) There is even an egg in one small duct in such a liver and there is no reaction. (Slide 11). In a few livers the inflammatory reaction in small portal areas is much more striking than the reaction around large fluke-containing ducts. (Slide 12) The great preponderance of eosinophiles in this small portal area suggests that an allergic reaction may in part account for the changes in ducts which appear to be much too small for adult flukes. Notice that there is also a proliferation of small bile ducts.

(Slide 13) The infiltrates in portal areas and the proliferation of fibrous tissue markedly accentuate the lobular pattern in some livers and (Slide 14) result in even greater disturbance of the lobular architecture in peripheral parts of the livers. (Slide 15) In a few livers there are masses of amorphous eosinophilic material of unknown nature distending the ducts. Finally, in some livers (Slide 16) very dense scar tissue and little inflammatory reaction is present around large bile ducts (Slide 17) Even in small portal areas very heavy bundles of collagen surround the ducts.

The changes we have observed in these cats closely parallel the changes I have seen in over 20 humans autopsies we have done at a provincial hospital in Thailand. The changes are similiar to those described in human Clonorchis sinensis infection by Hou Pao-Chang and to those of Opisthorchis felineus infection.

Especially in northeastern Thailand, liver disease is very prevalent. Exactly how much there is, is not known. Infestation by liver flukes undoubtedly causes disease but the actual morbidity is difficult to determine because it is not known how much liver damage is accounted for by infectious hepatitis, amebiasis, leptospirosis, malnutrition, alcoholism, plant toxins or genetic diseases such as hemoglobin E disease.

Table I

Cat No.	Estimate * No. of flukes from egg counts	Estimate No. of flukes Histologically	Degree of Scarring of Large Ducts	Weight Grams	Length Centimeters	Incidental Findings
1	4	Very few	±	810	33	
2	20	++	++	820	33	
3	60	+	+	1280	38	Myocarditis
4	40	++	++	1220	45	Lung nematode *Paragonimus* sp. *Leptospira javanica*
5	30	++	±	1320	35	Myocarditis
6	5	Very few	+	950	34	Lung nematode
7	10	+	+	660	29	
8	30	+	+	850	33	Myocarditis
9	10	+	+	1100	34	
10	30	Few	++++	1840	44	*Paragonimus* sp.
11	200	++	+++	2180	42	Lung nematode

* To nearest 10 except in livers estimated to have less than 10