Physaloptera clausa, a Possible New Reservoir Host for Parasitic Leptospires *

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The important role of many wild and domestic species of mammal in the epidemiology of leptospirosis is well known.[a] Recently, poikilotherms such as snakes were also incriminated as carriers of pathogenic leptospires.[b] Although the main vector hosts may vary from one region to another, rodents are thought to be the most important world-wide reservoirs for the maintenance of leptospirosis in nature. In spite of this, it is very important to recognize all other hosts, since changes in ecological conditions may also change the order of importance of hosts. The possibility of blood-sucking parasites, whether external or internal, serving as transmitters is an important problem to be faced, and the need for further research on it has been stressed.[a] Although external parasites have been described as playing a role in the epidemiology of leptospirosis,[a] there appears to be no incriminating evidence in the literature of any blood-sucking intestinal parasites, whether as " dead-end " or as reservoir hosts for pathogenic leptospires.

Materials and methods

Hedgehogs are considered to be important carriers of pathogenic leptospires in Israel,[c] and for this reason a constant check is kept on the percentage of carriers and the serotypes they harbour. The routine checking of infected wild field animals includes a postmortem examination for gross pathological lesions. If such lesions are observed, the affected organs are examined histologically. Routine staining of histological sections is done with haematoxylin–eosin (HE) and *p*-aminosalicylic acid (PAS) for pathological changes, and with Warthin's stain [d] for leptospires. Blood is taken for serological testing. Kidney and brain tissues are cultured in Korthof medium containing 8% rabbit serum. Fourteen different serotypes and subserotypes of leptospires are used in the microscopic agglutination test.

In addition to the routine examination, in the case of hedgehog No. 362 described in this Note, tissue sections were examined by a specific fluorescent-staining procedure in order to confirm and specifically identify the leptospires observed by the silver staining method. Tissue sections of organs previously kept in 10% formol were prepared for fluorescent staining by the method described by Maestrone.[e] Staining was done by the indirect immunofluorescent technique.[f] The serum of a human patient infected with the same serotypes of leptospires as the hedgehog was used for the fluorescent technique. The fluorescein-labelled serum was rabbit anti-human globulin. Specific inhibition controls were obtained by diluting the positive human serum with the homologous *Leptospira* culture that was agglutinated both by this serum and by the serum of the hedgehog.

Investigation and results

Autopsy findings. Hedgehog No. 362 (female) was thin and in poor general condition (weight, 560 g). The stomach was filled with round worms (Fig. 1), identified by Professor Witenberg as *Physaloptera clausa*. Many worms were free in the lumen, but many others were attached to the wall of the stomach. The ileum and colon contained similar nematodes. In the urogenital system, macroscopically the kidneys were slightly swollen, tense and dark; the bladder contained some orange-coloured urine. No significant pathology was seen macroscopically in other organs.

Histology of the stomach, ileum and colon showed essentially similar changes. The mucosa, submucosa, muscularis mucosae, and in some parts all the coats including muscularis, were infiltrated with acute, subacute and chronic inflammatory cells, among which eosinophils were prominent. Sections of

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[a] *Bull. Wld Hlth Org.*, 1965, **32**, 881-891.

[b] Turner, L. H., Bennett, L., Elisberg, C. E., Smith, G. & Broom, J. C. (1959) *Med. J. Malaya*, **14**, 83-98.

[c] Hoeden, J. van der (1958) *Trop. geogr. Med.*, **103**, 225-237.

[d] Lilli, R. D. (1954) *Histopathologic technique and practical histochemistry*, New York, McGraw-Hill, p. 401.

[e] Maestrone, G. (1963) *Canad. J. comp. Med.*, **27**, 108-112.

[f] Weller, T. H. & Coons, A. H. (1954) *Proc. Soc. exp. Biol. (N.Y.)*, **86**, 789-794.

FIG. 1

PHYSALOPTERA CLAUSA IN ILEUM OF HEDGEHOG NO. 362

FIG. 2

SECTION OF WORM IN STOMACH MUCOSA (HAEMATOXYLIN-EOSIN STAINING: ORIGINAL × 160; PHOTOGRAPH × 960)

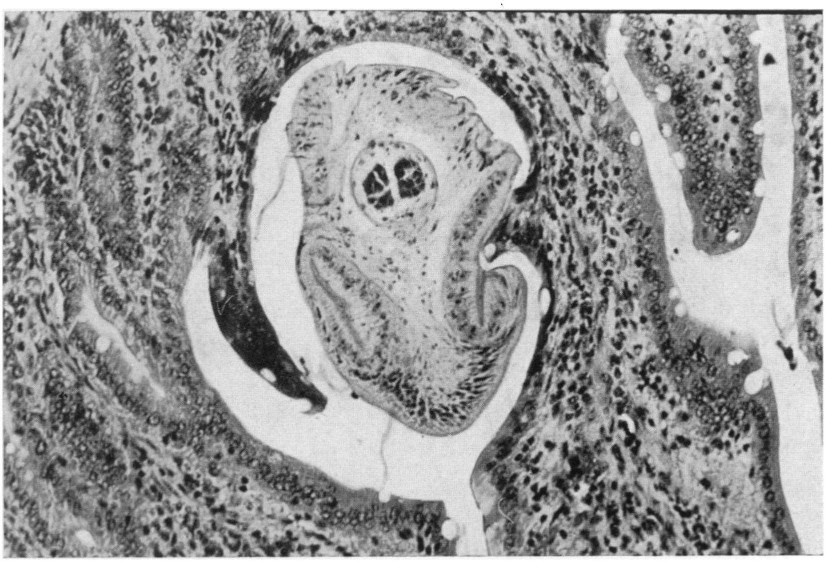


FIG. 3

LEPTOSPIRES IN WORM (WARTHIN STAINING: ORIGINAL × 1000; PHOTOGRAPH × 3000)

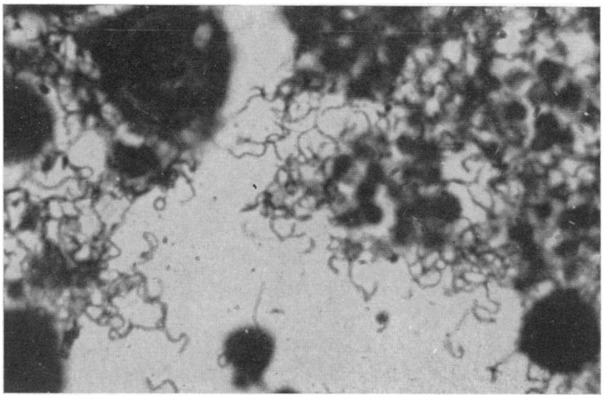
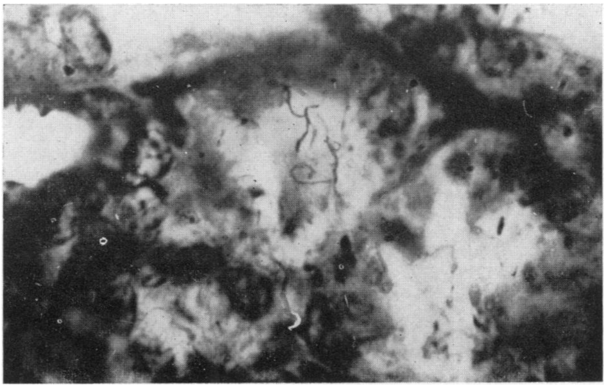


FIG. 4

SECTION OF KIDNEY WITH LEPTOSPIRES (WARTHIN STAINING; ORIGINAL × 1000; PHOTOGRAPH × 3000)



worms were seen in the mucosa of various parts of the alimentary tract (Fig. 2).

Warthin staining revealed very large numbers of structures resembling leptospires, mainly in the coelomic cavity of the worms (Fig. 3). Confirmation that these were indeed leptospires was obtained by specific fluorescent staining.

Histology of the kidney showed small areas of interstitial inflammation. These changes may often be found in the later stages of leptospiral infection, when cultures are already negative. Silver staining showed the presence of leptospires (Fig. 4). This again was confirmed by specific fluorescent staining.

Serological findings. The sera of the hedgehog agglutinated leptospires belonging to the Hebdomadis group—*hebdomadis hebdomadis*, *mini szwajizak* and *sejroe sejroe*—up to a titre of 1:2000.

Cultural findings. Leptospira cultures from kidney, urine and brain were negative.

Discussion

The finding of leptospires within the coelomic cavity of a blood-sucking internal parasitic worm does not appear to have been recorded previously. It seems probable that, if such blood-sucking parasites were present in the intestine during the leptospiraemic stage of the mammalian host, they could easily become infested. The finding of numerous leptospires within the worms in the present case seems to indicate that leptospires multiply in the coelomic cavity rather than just pass through. The worms were identified as being the nematodes *Physaloptera clausa*. The life-cycle of this worm is not well-established. It is known that arthropods such as beetles and cockroaches serve as intermediate hosts for the development of the worms. The worms become attached to the mucosa and are blood suckers. They are also known to change their position occasionally, leaving numerous wounds that may continue to bleed and become further contaminated and infected, thus causing excessive erosion and inflammation of the mucosa.

Conclusions

The finding of large numbers of leptospires within the parasitic worms was unexpected. If such mature worms are released via the bowel, they may serve as vectors of leptospirosis, contaminating the soil and infecting domestic animals.

The question whether *Physaloptera clausa* is a "dead-end" host for *Leptospira* or a potential vector has yet to be resolved.

The finding of leptospires in histological sections of organs from which leptospires could not be isolated is a common occurrence. The difficulty in isolating leptospires from infected organs that is experienced even by well-trained technicians makes it essential that histological sections be examined both by silver staining and by a specific fluorescent-staining technique.

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