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PARASITIC LIVER LESIONS IN SWINE, EXPERIMENTALLY PRODUCED BY VISCERAL LARVA MIGRANS OF TOXOCARA CATI

By
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The liver lesions in swine, usually called hepatitis interstitialis chronica parasitaria multiplex, white spots, or parasitic scarring, is of very common occurrence and has been known for some time. The changes have been described macroscopically as grey-white spots with ill-defined borders and were most often considered to be scar formations. In German literature the cause of this liver damage was for many years attributed to, inter alia, invasion of Cysticercus tenuicollis, but nowadays, the cause is generally considered to be larval migration of *Ascaris suum*.

Contrary to this assumption, however, is the observation that certain pigs can have an abundance of *A. suum* in the intestine, while the liver is practically free from parasitic damage, and on the other hand, pigs with numerous parasitic scars in the liver can completely lack this parasite in the intestine. Furthermore it was later noticed that certain nematode larvae were able to migrate in unnatural hosts and there give rise to the clinical syndrome described in children as visceral larva migrans in which liver damage is a principal symptom. These facts stimulated the author's interest in finding out if any ascarid foreign to swine and commonly occurring in their environment, could produce liver lesions of the type termed "white spots."

Among the possibilities for visceral larva migrans which may occur in the pig, the most probable is the invasion of *T. cati* larvae. Cats are very common in Swedish swine stalls, and are often

infected with *T. cati*. Besides, as cats leave their droppings in swine stalls, outside in the runs, in grainstores among the loose grain and feed, and in soil intended for small pigs to eat, it is very probable that pigs are exposed to invasions by this parasite.

In order to ascertain whether a larval migration of *T. cati* can occur in pigs, and whether liver damage similar to that seen in the livers of slaughter swine can be produced in this way, the following experiment was undertaken.

MATERIAL AND METHODS

The *experimental swine* used were Swedish Landrace, born in the institution's stalls in the first litters of two gilts. At repeated examinations of the faeces the gilts were free from *Ascaris* eggs, and as a further precaution, the pigs were given a course of piperazine before farrowing. The experimental pigs were born in carefully disinfected boxes. At the beginning of the experiment the pigs which had been kept strictly isolated since birth were weaned. They were between four and five weeks old and weighed an average of 8 kg.

For the *collection of eggs,* the uteri of *T. cati* females were dissected out, and fertilised eggs with shells were collected in large petri-dishes and washed in ordinary tap water. The water was 0.5 cm. deep and no antiseptic agent was added. For the embryonation of the eggs, the petri-dishes were kept at room temperature. Every day the lid was removed for a few hours and the contents carefully shaken, so that the developing eggs would get a sufficient supply of oxygen. After five weeks, a well developed larva had developed in most of the eggs. The embryonated eggs were counted with the aid of a *McMaster* chamber. With a pipette 0.5 cc. of the shaken egg suspension was transferred to 19.5 cc. concentrated saline solution. The three counting chambers were filled with a part of this mixture, after which the number of embryonated eggs per unit volume was calculated in the original suspension.

The required number of eggs were mixed in about 30 ml. of water and administered with a stomach tube. Owing to the strongly adhesive nature of the eggs, the funnel and tube were rinsed with about the same amount of water.

Two of the experimental pigs (no. 1 and 2) received approx. 35,000 eggs, two pigs (no. 3 and 4) approx. 20,000 and four pigs (no. 5, 6, 7 and 8) approx. 100,000 eggs. Three pigs (no. 9, 10

and 11) were not treated and kept as controls. At the time the eggs were given, the control pigs were transferred to a separate isolated box. Pigs numbers 1 and 2 and control pig 9 were killed after three days, numbers 3, 4, 5, 6 and control pigs 10 and 11 were killed after 10 days, while numbers 7 and 2 were not killed until 63 days. The planning of the experiment is shown in table 1.

Table 1.

Experimental pig no.	Number of *T. cati* eggs administrated	Days between administration of eggs and killing
1	35.000	3
2	35.000	3
3	20.000	10
4	20.000	10
5	100.000	10
6	100.000	10
7	100.000	63
8	100.000	60
9	0	10
10	0	10
11	0	3

The pigs were autopsied while still warm. Representative parts of different organs were fixed in neutral 10 % formaldehyde solution for histological examination. The histological sections were stained routinely with hematoxylin and eosin and with PAS according to *Hotchkiss*. In some cases the sections were also stained with *v. Gieson, Gomori*'s silver impregnation, iron staining by *Turnbull*'s blue method after *Tirman Schmelzer,* plasma cell staining according to *Kurnick,* 1 % toluidin blue in 60 % alcohol and with PAS after exposure to 1 % maltdiastase.

The liver, lungs and intestine were subjected to parasitological investigation.

Immediately before destruction, blood tests were made for differential counts of the white corpuscles.

RESULTS

Throughout the time of the experiment, no noteworthy signs of illness or disturbed general condition were shown, apart from the fact that, about one week after the egg administration, the experimental pigs coughed somewhat if chased around in the box.

Macroscopic changes:

Infection with *T. cati* eggs caused different macroscopically observable lesions in the internal organs of the experimental pigs. The morphological picture was similar in those pigs killed after the same period following the egg administration, but there was a marked difference between the lesions in pigs killed on the 3rd, 10th, and 63rd respectively after the infection with the eggs.

The liver: In the pigs killed three days after the 35,000 *T. cati* eggs were given, the surface of the liver was entirely covered with small, recent haemorrhages, about 1 mm.^{2} in size, and star-shaped grey-white spots of similar size, in which the interlobular connective tissue seemed to be thickened (Fig. 1). Similar changes were also found spread diffusely throughout the interior of the organ.

In the pigs killed 10 days after the introduction of the 20,000 and the 100,000 eggs respectively, the haemorrhages had completely disappeared. The grey-white spots on the other hand, had increased in size and were about 3 mm. in diameter (Fig. 2). The spots seemed to consist of a network of thickened interlobular septa. These thickened septa seemed to radiate out both superficially and deeply from the damaged area, which gave the spots a blurred appearance. Similar spots were also found in the interior of the liver, but were there more difficult to discover. A quantitative difference in the number of spots existed between the pigs given 20,000 eggs and those given 100,000. There was in fact a decidedly smaller number of spots in the two pigs which received the smaller dose.

The third group of pigs, the two killed after 63 days and which received about 100,000 *T. cati* eggs each, presented an almost negative picture (Fig. 3). Only isolated and rather faintly appearing grey-white spots were found on the surface of the liver, and internally, only one similar but more compact spot was observed. No increase in the consistency of the liver could be established with certainty in the experimental pigs.

Other organs: The mesenteric lymph nodes of the small intestine were distinctly swollen, especially in the pigs killed on the third and tenth days.

The lungs of the pigs killed on the third day were macroscopically without any remarkable changes, while the lungs of pigs killed on the tenth day were moderately emphysematous and somewhat oedematous. Subserously and also in the lung paren-

chyma were many petechial haemorrhages and grey-white relatively well defined areas of the size of a pin head. Similar spots were also found isolated in the pigs killed on the 63rd day.

The kidneys on the tenth day showed small, isolated, subcapsular, grey-white spots.

The control pigs exhibited no changes indicative of parasitic lesions in either liver, lungs or other organs.

Microscopic changes:

The microscopic findings confirmed the macroscopic findings, in that the experimental pigs given *T. cati* eggs and killed at the same time as each other, all showed a uniform picture regarding the changes, while there was a clear difference between the lesions in those killed at different times after egg administration.

The liver: In the two pigs killed on the *third day,* individual *T. cati* larvae were encountered in sections from the liver. The larvae which in the liver sections had a diameter of 14 μ, were difficult to observe in sections stained with hematoxylin and eosin, but could be more easily found in a PAS stained section, through their PAS positivity (Figs. 4, 5, and 6). In all cases, the larvae lay intralobularly in the lobules where haemhorrhages and cell infiltrations were also present, but a little apart from these areas of lobular lesions they lay in the sinusoids without a surrounding tissue reaction.

The small, macroscopically observable haemhorrhages lay intralobularly (Figs. 7 and 8). The haemhorrhages did not appear to have any particular localisation within a liver lobule. In shape they were irregular and their formation gave the impression of having followed the larva's migration within the lobule. In the central part of the haemhorrhages there were generally found necrobiotic and necrotic liver cells with acidophilic cytoplasm. Nuclear changes were pyknosis, karyorrhexis and karyolysis (Fig. 8). In the haemhorrhages there were often found isolated eosinophil and neutrophil leucocytes.

The grey-white spots, macroscopically observable on the third day, consisted of severe, focal intralobular cell infiltrations and also of extensive cellular infiltrations in the adjacent interlobular septa. The infiltrating cells consisted principally of histiocyte cells, with big, light nuclei, and of a smaller number of eosinophil leucocytes (Figs. 4, 9, 10 and 11).

Within the lobules where the fresh parasitic lesions were

found, the surrounding, apparently uninjured liver cells had a sharp decrease in the glycogen content which was clearly demonstrated in PAS staining. The same condition was also present in the adjacent parts of surrounding lobules (Fig. 4). Within the same area, the cytoplasm of the liver cells were strikingly silver positive by means of the Gomori's method (Fig. 12) and slightly eosinophilic.

In the four pigs killed on the *tenth* day, a striking change in the liver damage had occurred. Small numbers of larvae were also present here. The intralobular haemorrhages had disappeared even microscopically, and no trace of them in the form of haemosiderin could be found with iron staining. The liver necrosis which arose in connection with the haemorrhages had also disappeared.

The lack of glycogen and the silver positivity in the cytoplasm of the liver cells around the site of the parasitic lesions, observed in the pigs killed on the third day, had almost completely disappeared and remained in part in only isolated lobuli.

The macroscopically observable grey-white spots still consisted of principally intra- and interlobular cell infiltrations, but the cells at this time were predominately eosinophil leucocytes (Figs. 13, 14, 15 and 16), while on the other hand, the histiocytes had decreased in number. In these pigs, the histological picture was dominated by the fact that the interlobular and periportal connective tissue in the affected area was greatly thickened by an enormous infiltration of predominantly eosinophil leucocytes (Figs. 15 and 16). Only an insignificant increase in the collagenous connective tissue was shown with *v. Gieson* staining.

In the two pigs killed on the *63rd day,* there remained isolated grey-white spots, most often consisting of fibrously thickened interlobular connective tissue septa with a moderate admixture of eosinophil leucocytes (Figs. 17 and 18). The more compact macroscopically observable areas in the liver parenchyma proved to be composed of moderately well developed follicle-like lymphocyte aggregations, situated periportally and surrounded by a thin connective tissue capsule (Fig. 19). In the adjoining interlobular septa there was an increase in the connective tissue with a moderate number of eosinophil leucocytes. In other parts of the liver, the great number of eosinophils which were found ten days after the introduction of the eggs, had entirely disappeared, and no other obvious traces of the larval migration could be found. Lo-

cal liver cell regeneration was present in a lobule of one of the subserous pathological areas (Figs. 17 and 18). Here, the liver cells were enlarged and sometimes bi-nucleate and the cytoplasm was poor in ribonucleotides.

Other organs:

The *mesenteric lymph nodes* along the duodenum, jejunum and ileum were the organs in which *T. cati* larvae were most numerous.

In the pigs killed on the third day, the larvae occurred most frequently, and often several were present in the same section. Frequently no tissue reaction was present around the larvae (Fig. 20), but sometimes isolated eosinophil leucocytes were in the vicinity (Fig. 21). Moderate oedema occurred in the mesenteric lymph nodes, and at this stage there was only a moderate, diffuse eosinophilia. Abundant multi-nucleate giant cells were present in the cortex, where they were often in association with small necrotic foci.

On the tenth day, the picture was dominated by a very severe and widespread, diffuse infiltration of eosinophil leucocytes, while around the larvae were, in most cases, large aggregations of eosinophils. The number of giant cells had decreased.

On the 63rd day, only isolated larvae could be found. Their cuticles appeared shrunken, and they were only weakly PAS positive. They were probably dead and were surrounded by a small amount of eosinophilic matter and some eosinophil leucocytes (Fig. 22). In other areas, the eosinophil leucocytes in the lymph nodes were reduced to normal. No giant cells could be observed in the lymphatic tissue.

The lungs: Initial irritation of the lung tissue occurred after three days. In the interstitium, there was a slight increase in the histiocytes with some isolated eosinophil leucocytes.

After ten days, many *T. cati* larvae were found in the lungs. In the interlobular connective tissue septa of the lungs and in the peribronchial fibrous tissue were many extensive focal infiltrations of eosinophil leucocytes.

After 63 days, no larvae were present in the sections. The areas and the infiltrations of eosinophil leucocytes had disappeared. Only a few well defined granulomatous formations remained in the lung parenchyma at the sites of earlier reactions.

The kidneys: The small subcapsular spots observed in the

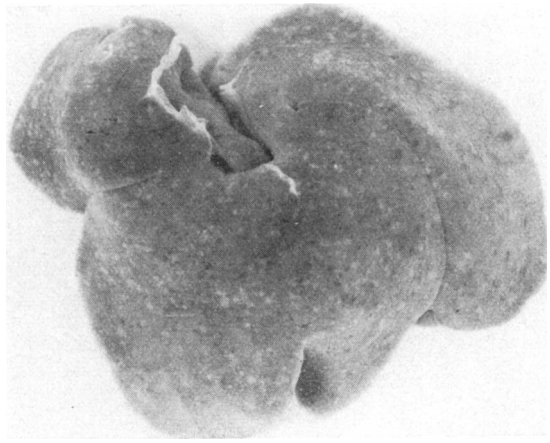

Fig. 1. O 4166-62. Liver 3 days after the administration of approx. 35,000 *T. cati* eggs. Many subserous haemorrhages, about 1 mm.^{2} in size, and also star-shaped, grey-white areas.

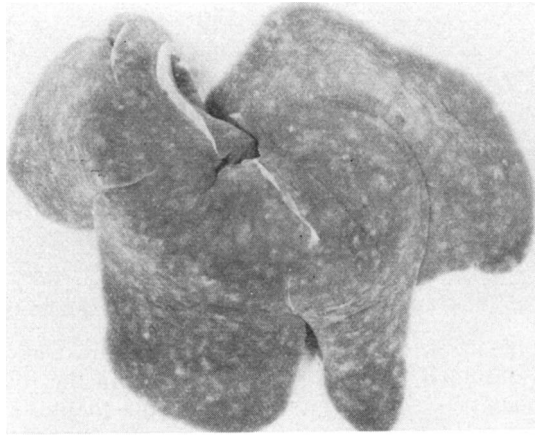

Fig. 2. O 7826-61. Liver 10 days after the administration of approx. 100,000 *T. cati* eggs. Many subserous, grey-white, star-shaped areas, consisting of a network of thickened interlobular connective tissue septa. The diameter of the areas is about 3 mm. The haemorrhages are no longer present.

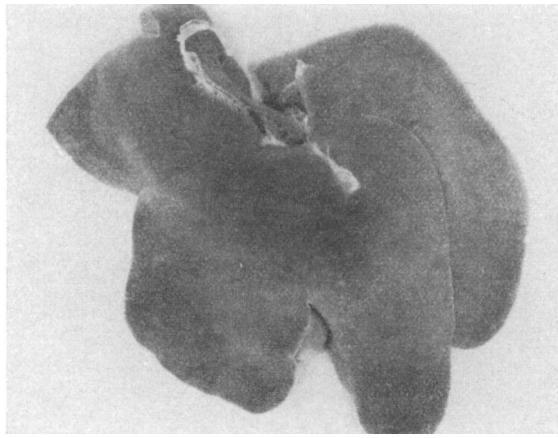

Fig. 3. O 795-62. Liver 63 days after the administration of approx. 100,000 *T. cati* eggs. Isolated, subserous, grey-white areas.

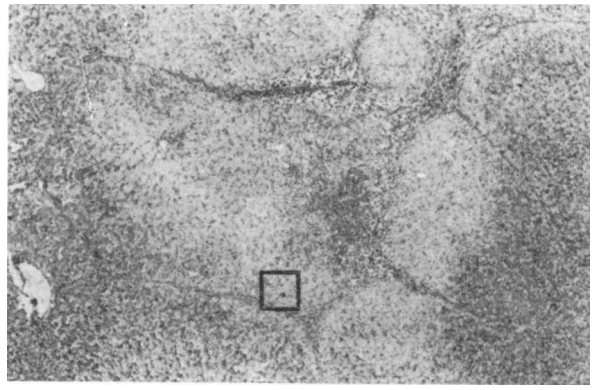

Fig. 4. O 1466-62. Liver 3 days after the administration of *T. cati* eggs. A cross-sectioned *T. cati* larva is seen within the square without surrounding cellular reaction. Within the same lobule, above and to the right of the square, liver necrosis is seen with cell infiltration. The adjacent interlobular connective tissue septa are thickened by cell infiltration. PAS staining demonstrates a sharply decreased glycogen content in the liver cells within a damaged lobule and also in parts of the adjacent lobules, in connection with this recent liver damage. PAS 40 ×.

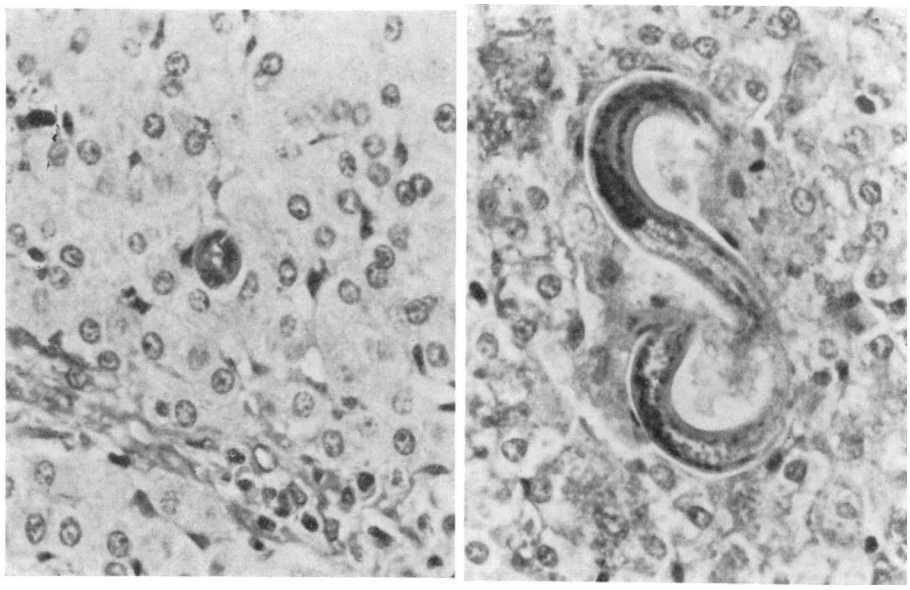

Fig. 5. O 1466-62. Detailed picture of Fig. 4. The cross-sectioned *T. cati* larva lies without surrounding reaction. The ala formation, appearing as a faintly visible spine is seen on the lower edge of the sectioned larva. PAS 400 ×.

Fig. 6. O 1466-62. Part of the liver, 3 days after the administration of *T. cati* eggs. A longitudinal section of a *T. cati* larva lying without surrounding reaction in a sinusoid. The larva lies in a sinusoid with clearly visible sinusoidal endothelium. PAS 400 ×.

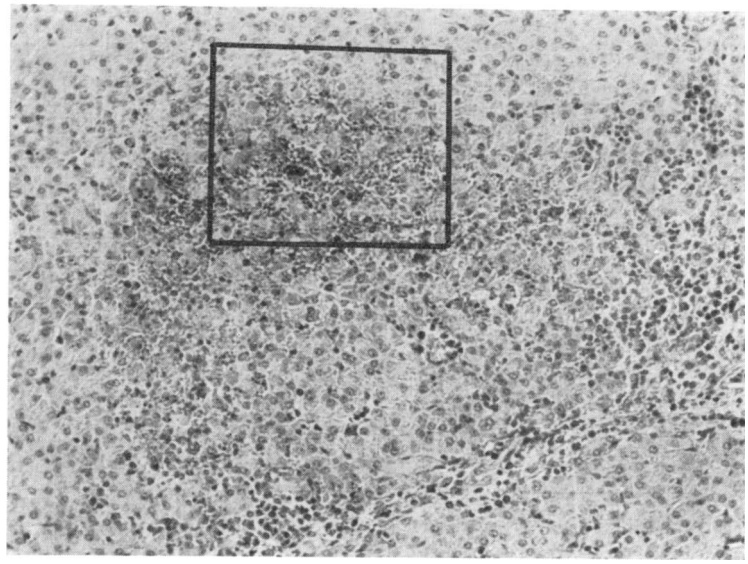

Fig. 7. O 1466-62. Liver 3 days after the administration of *T. cati* eggs. Intralobular areas consisting of fresh haemorrhages. The adjacent interlobular septa are thickened owing to infiltration, principally of histiocytes and some eosinophil leucocytes. Htx-eosin, 50 ×.

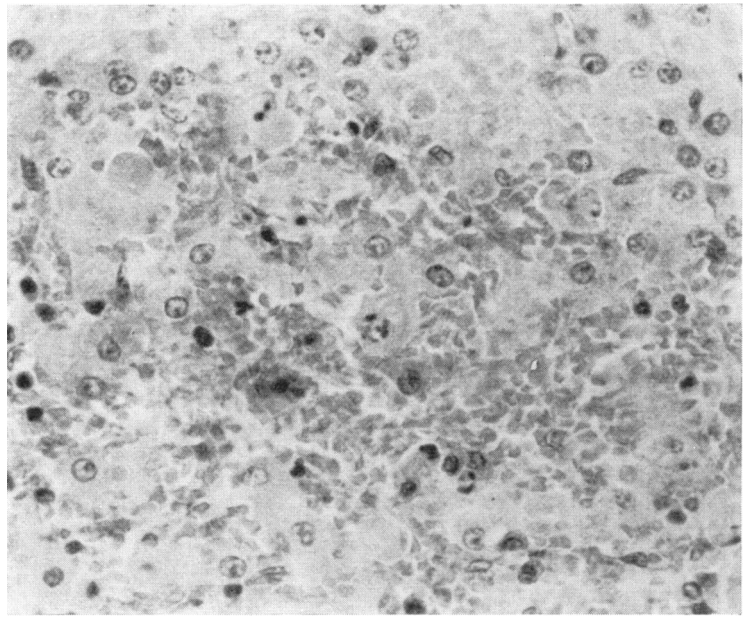

Fig. 8. O 1466-62. Detailed picture of the enclosed area of Fig. 7. Intralobular haemorrhages and degenerative changes in the liver cells with karyorrhexis, pyknosis and karyolysis. Htx-eosin, 500 ×.

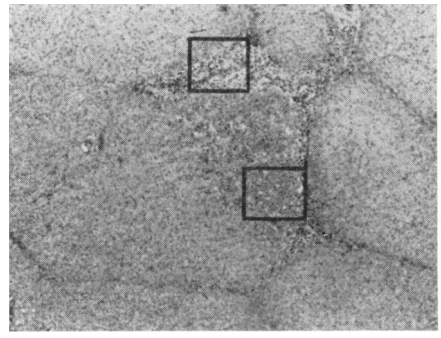

Fig. 9. O 1466-62. Liver, 3 days after the administration of *T. cati* eggs. The same case as in Fig. 4. The upper square shows interlobular connective tissue, infiltrated with histiocytes and eosinophil leucocytes, and the lower one, intralobular liver necrosis with infiltration of predominantly histiocytes. Htx-eosin, 40 ×.

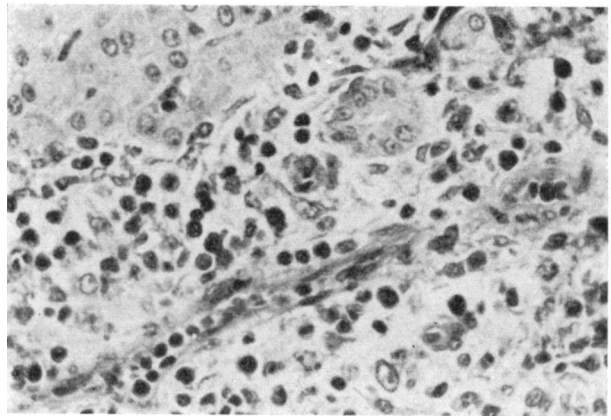

Fig. 10. O 1466-62. Detailed picture of Fig. 9 (upper square). Many histiocytes with large light nuclei and eosinophil leucocytes with dark nuclei in the interlobular connective tissue. Htx-eosin, 400 ×.

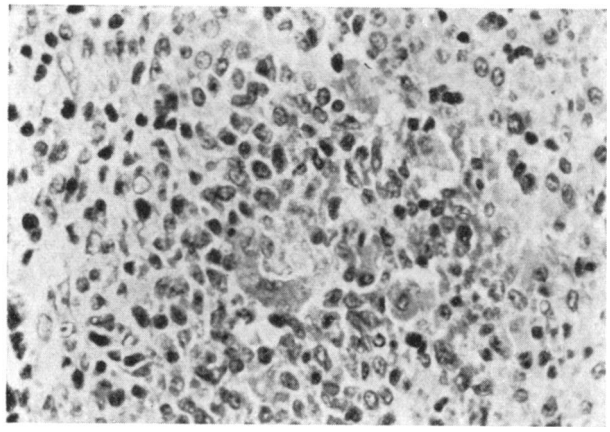

Fig. 11. O 1466-62. Detailed picture of Fig. 9 (lower square). Intralobular liver necrosis with a small haemorrhage. The infiltration consists predominantly of histiocytes with large, light nuclei, and isolated eosinophil leucocytes with dark nuclei. Htx-eosin, 400 ×.

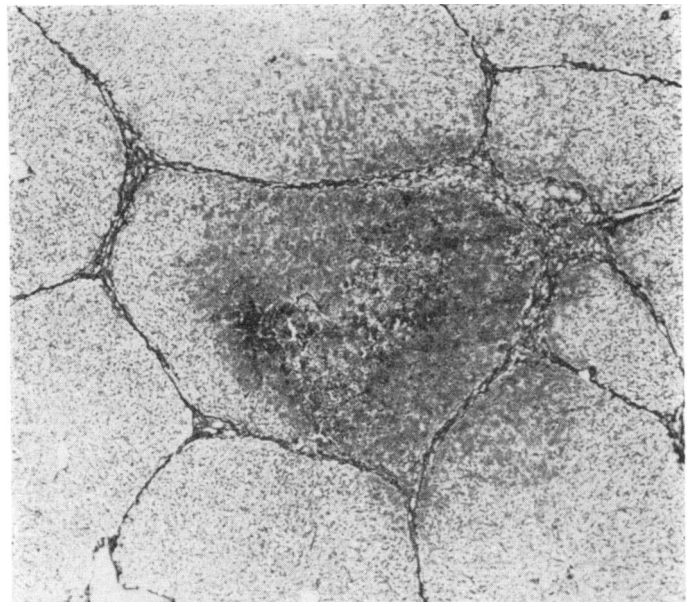

Fig. 12. O 1466-62. Liver 3 days after the administration of *T. cati* eggs. The same case as in Figs. 4 and 9. The glycogen free liver cells around the liver necrosis are strongly silver positive in the cytoplasm. Gomori's silver stain, 50 ×.

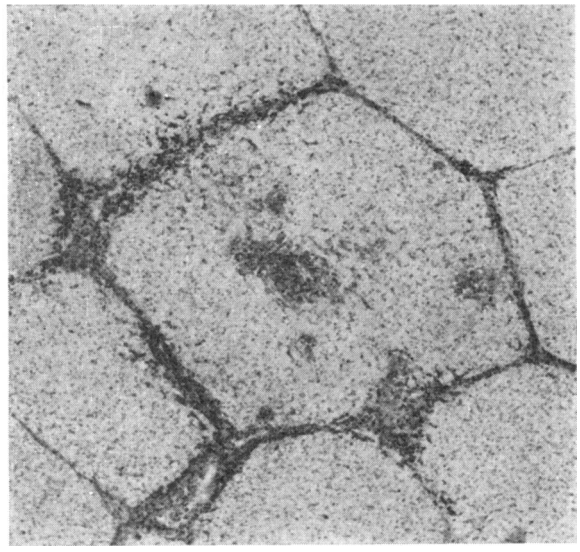

Fig. 13. O 7826-61. Liver 10 days after the administration of *T. cati* eggs. Several small intralobular areas consisting mainly of eosinophil leucocytes. The area in the middle of the lobule contains a section through a *T. cati* larva. The surrounding interlobular connective tissue is thickened with a large number of eosinophil leucocytes. Htx-eosin, 50 ×.

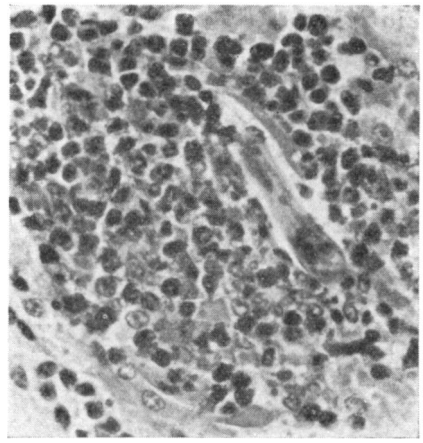

Fig. 14. O 7826-61. Detailed picture of Fig. 13. A sectioned *T. cati* larva, surrounded by eosinophil leucocytes. Htx-eosin, 400 ×.

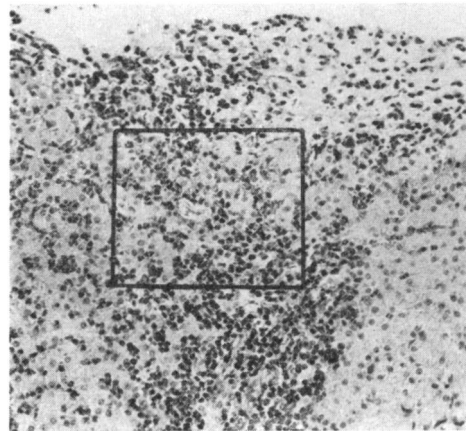

Fig. 15. O 7853-61. Liver 10 days after the administration of *T. cati* eggs. Part of a superficial parasite spot. The interlobular septa is greatly thickened and heavily infiltrated with eosinophil leucocytes. A *T. cati* larva (in the square) is situated intralobularly and surrounded by eosinophils. Htx-eosin, 120 ×.

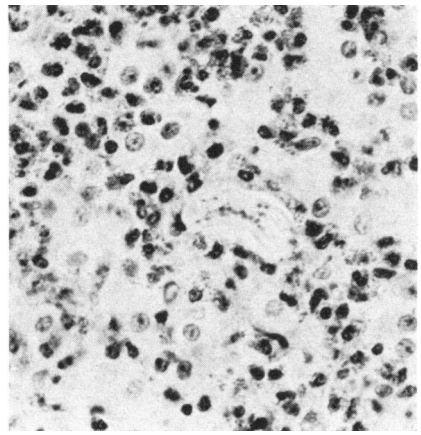

Fig. 16. O 7853-61. Detailed picture of Fig. 15. A *T. cati* larva surrounded by a large number of eosinophil leucocytes lies in the centre. Htx-eosin, 500 ×.

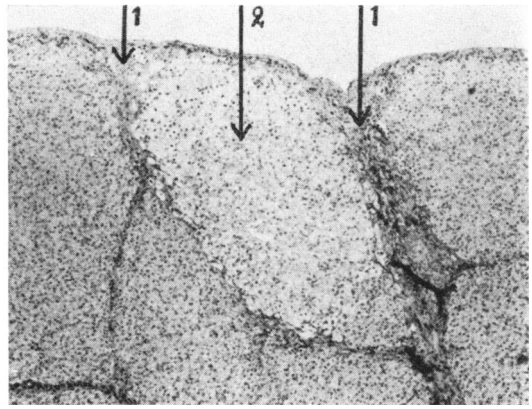

Fig. 17. O 795-62. Liver 63 days after the administration of *T. cati* eggs. Thickened interlobular septa are seen situated superficially in the liver parenchyma (1). The thickening is caused by an increase in fibrous connective tissue, poor in cells, with a moderate number of eosinophil leucocytes. The centre lobule (2) is a site of liver cell regeneration. Htx-eosin, 40 ×.

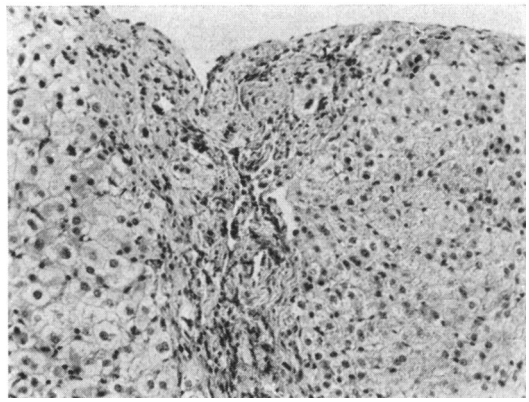

Fig. 18. O 795-62. Detailed picture of Fig. 17 showing the thickened, superficial interlobular connective tissue septa on the right. In the septa there is a proliferation of fibrous connective tissue, poor in cells, with a moderate admixture of eosinophil leucocytes. To the left is a regenerated liver lobule, whose cells are enlarged, poor in ribonucleotides and sometimes binucleate. Htx-eosin, 120 ×.

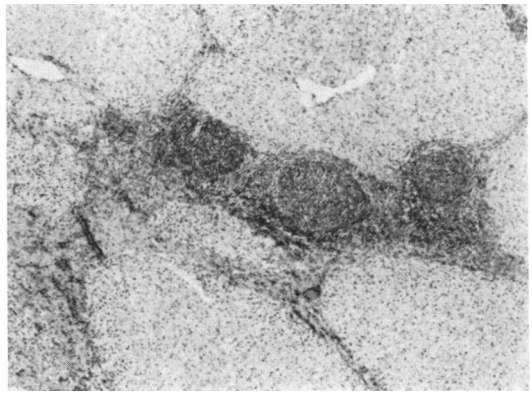

Fig. 19. O 765-62. Liver 63 days after the administration of *T. cati* eggs. Compact area deep in the liver parenchyma. It is composed of follicle-like lymphocyte aggregations, situated in the periportal connective tissue. In the adjacent interlobular septa is an increase in the connective tissue in which there are a moderate number of eosinophil leucocytes. Htx-eosin, 40 ×.

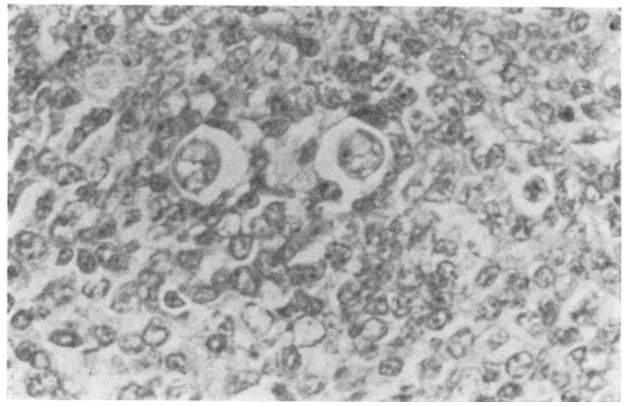

Fig. 20. O 1466-62. Mesenteric lymph node 3 days after the administration of *T. cati* eggs. 3 cross-sections of *T. cati* larvae. No surrounding reaction. The alae formations of the larvae are seen in the cross-section on the far left. Htx-eosin, 500 ×.

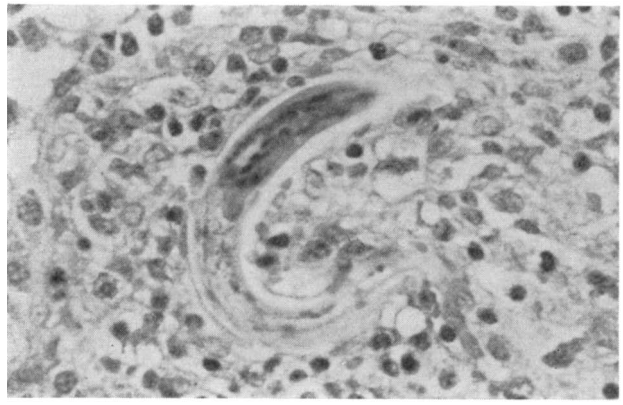

Fig. 21. O 1465-62. Mesenteric lymph node 3 days after the administration of the *T. cati* eggs. Longitudinal section of a *T. cati* larva with isolated eosinophil leucocytes in the surrounding tissue. Htx-eosin, 500 ×.

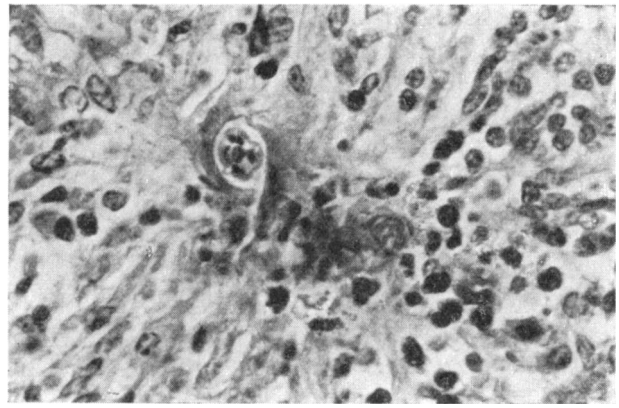

Fig. 22. O 795-62. Mesenteric lymph node 63 days after the administration of *T. cati* eggs. A cross-sectioned larva lays in the centre of the picture. The cuticle of the larva is shrunken and surrounded by a small amount of eosinophilic matter and a number of eosinophil leucocytes. This larva was evidently dead. Htx-eosin, 500 ×.

kidneys concisted principally of infiltrations of eosinophil leucocytes.

The stomach and the intestine: In the submucosa of the small intestine of the pigs killed on the third and the tenth days, small isolated focal infiltrations of eosinophil leucocytes were found. No such infiltrations were observed in the mucous membrane of the stomach.

Parasitological investigations

On parasitological investigations larvae were found in scrapings taken from the livers of experimental pigs, killed on the third and the tenth days. Larvae were also found in the lungs in pigs killed on both the third and on the tenth days. No parasites, however, were found in the liver or lungs of pigs, killed on the 63rd day. Parasitological examination of the intestines gave negative results in all the pigs.

Examination of the blood

The differential counts of the white blood cells revealed, that the relative numbers of eosinephil leucocytes in the pigs killed on the third day after experimental infestation, was on the aver-

age 3.5 %, while in those killed on the tenth day, the average was 15.6 %, and in those killed on the 63rd day, 2.5 %. The control pigs had an average of 1.5 % eosinophil leucocytes.

DISCUSSION

In recent years it has been increasingly observed that some ascarids, expecially those of the dog and the cat, can migrate as larvae in host animals foreign to the respective parasites without reaching sexual maturity. The migration of these larvae causes damage in different tissues, especially the liver and the lungs. Thus, *Beaver et al.* (1952) found that the intestinal parasite of the dog *Toxocara canis,* can in children cause the disease complex called visceral larvae migrans. *Beaver* and co-workers regarded visceral larva migrans as a relatively benign disease, its principal characteristics being a sustained eosinophilia, pneumonitis and hepatomegaly with granulomatous lesions in the liver. A larval nematode was observed in sections from the liver of one patient and was identified as *Toxocara canis* or possibly *Toxocara cati.* In a later paper, *Beaver* (1956) wrote that *T. canis* as well as *T. cati* could cause this syndrome, which in isolated cases led to death. *Braun* (1961) observed that ascarids of the dog and the cat could cause visceral larva migrans in children, most often between the ages of 18 month and four years. This author considers that the disease complex is not sufficiently well known.

Several authors have shown experimentally that visceral larva migrans can be produced in experimental animals with different ascarids. Thus, *Fülleborn* showed as early as 1921 that the feeding of *Ascaris lumbricoides* eggs to guinea pigs was followed by larval migration in different organs. *Sprent* (1951) infected mice with various foreign ascarid eggs, and found larval migration in different body tissues. *Tiner* (1951) described the migration of ascaris larvae of the racoon in rodents. In pigs, *Done et al.* (1960) showed experimentally that visceral larva migrans of *Toxocara canis* was possible. They investigated the possibility of larval migration to the central nervous system and observed then that the larvae migrated to inter alia the liver and that lesions in the form of spots was the results. They did not, however, closely relate their observations to the spontaneous appearance of "white spots" in the livers of swine.

When the *Ascaris* larvae migrate in a foreign host, the migration should be along the general lines it would take in its nor-

mal host. For many ascarids the path of the migration is considered to go from the intestine via the liver to the lungs and also by the lymphatic route directly to the lungs via *Ductus thoracicus* and from the lungs via the trachea and pharynx back to the intestine. Thus, the liver is considered in most cases to be included in this path of migration.

Regarding the normal invasion of *T. cati* in cats, *Sprent* (1956) considers that there are two possibilities. Mice can contain the second stage of the *T. cati* larvae. If the cat devours such larvae infested mice, these partly developed larvae only invade the mucous membrane of the stomach, and after cuticle changes migrate back to the digestive tract and there develope into mature individuals. In the second case, the invasion would be caused by the ingestion of embryonated *T. cati* eggs, and then the larval migration would be mainly via the lymphatic route to the lungs and thence to the digestive tract. Thus as a rule, there would be no passage through the liver.

The experiment described in this work showed that in pigs infected per os with embryonated *T. cati* eggs, the larvae appeared in the liver on the third day and caused a great deal of intralobular liver lesions with consequent cellular reaction in the interlobular connective tissue. At the same time a large number of larvae occurred in the mesenteric lymph nodes and later also in the lungs. This indicates that, in pigs, *T. cati* migrates in part via the portal system through the liver and in part, by the lymphatic route via *Ductus thoracicus* to the lungs.

This experiment has thus shown the possibility of larval migration of *T. cati* in swine.

The migration of *T. cati* does not seem to cause the same damage in the livers of cats, as in a host foreign to this nematode. *Sprent* considered, as was described earlier, that in cats, a larval migration to the liver occurred in only exceptional cases. Liver damage in this animal caused by *T. cati,* is apparently not described in the literature.

Parasitic liver damage in man, caused by invasions of *Toxocara* has been described in connection with biopsies and isolated *post mortems*. No morphological difference was given between the damage caused by *T. canis* and that caused by *T. cati.*

Beaver et al. (1952) found in man, through laparotomy and biopsy, that the lesions appeared on the surface of the liver as white areas (plaques), measuring 5—10 mm. in diameter. The

areas consisted of necrosis and extensive focal infiltrations of eosinophil leucocytes. Around some of the necrotic areas were eosinophil leucocytes and also many epitheloid cells and giant cells of a foreign body type. In one case, a number of *Toxocara* larvae were also observed. Liver damage in man caused by visceral larva migrans was also similarly described by *Dent* (1960). The lesions were in the form of round or linear grey-white granules, 2 to 5 mm. in diameter and apparently below the liver capsule. The histological structure of the granules consisted of central fibrinous necrosis, epitheloid cells and a large infiltration of eosinophils, lymphocytes and plasma cells. Older lesions showed varying amounts of fibrous tissue, foreign body giant cells, *Charcot-Leyden* crystals, epitheloid cells, macrophages and lymphocytes around the degenerate larvae. According to *Stowens* (1959), the liver damage was of two types; firstly a granulomatous reaction, later followed by a fibrous reaction. The granules were dominated by eosinophil leucocytes. During the healing process epitheloid and also giant cells appeared.

As the experiment described here showed that *T. cati* is able to cause liver damage in swine through larval migration, it remains finally to discuss whether this liver damage corresponds with that occurring spontaneously, the so called parasitic scars ("white spots") in the livers of swine.

This parasitic liver damage in swine is often mentioned briefly in modern text books of pathology. In older text books, it is described under the term "hepatitis interstitialis parasitaria multiplex" *(Joest* 1937). The same liver damage is also described by *Nieberle* and *Cohrs* (1962) under this heading, and they state it is present in 20—25 % of all swine. The liver damage was most often evident on the surface of the liver in the form of grey-white areas with a netlike structure. In the fresh stage, the areas consisted of fibroblastic, cell rich granulation tissue, with a strong infiltration of eosinophil leucocytes, and later, of fibrous connective tissue with a more or less pronounced tissue eosinophilia. The cause of this damage was said to be invasion of *Cysticercus tenuicollis,* or young liver flukes. *Schwartz* and *Alicata* (1932) believed that the parasitic lesions in swine livers always contained an increased amount of interlobular connective tissue, usually accompanied by an increased number of eosinophil leucocytes. They found larvae, which they diagnosed as *Ascaris suum* larvae and considered them to be the cause of the liver lesions.

White (1941) described parasitic liver damage in swine as "white spot liver". The lesions consisted of focal haemorrhages and degenerations, inflammations and fibrosis associated with a very heavy infiltration of eosinophil leucocytes. The authors considered that the damage was caused by migrating *Ascaris suum* larvae. *Oldham* and *White* (1942) produced parasitic liver lesions by giving large doses of embryonated *Ascaris* eggs (apparently *Ascaris suum*). They showed that fresh lesions consisted of focal haemorrhages and necrosis of the liver cells quickly followed by an intensive infiltration of eosinophils and proliferation of fibroblasts. Older lesions often displayed new formation of lymphoid tissue. Liver damage was accompanied by an increased number of eosinophils in the blood.

The liver lesions occurring in the present experiment, corresponded in certain cases both with those described in man in connection with visceral larva migrans of *Toxocara* invasions, and with the spontaneously appearing lesions in pigs.

It became clear from this experiment that the macroscopic appearance and histological structure of the liver lesions varied according to its age. Thus, a marked difference could be established between the lesions 3, 10 and 63 days old respectively.

The most severe liver damage was evident in those pigs killed as early as three days after the administration of the *T. cati* eggs. In this case, there were small grey-white spots and multiple, macroscopically observable minor haemorrhages. The haemorrhages were situated intralobularly in association with liver cell necrosis. *T. cati* larvae were also found in some of the injured lobules. All this indicates that these fresh lesions were a direct consequence of the mechanical effect of the larvae on the liver parenchyma. Changes in the surrounding liver cells, consisting of absence of glycogen, appearance of silver positivity and eosinophilia of the cytoplasm, indicate in addition, that all the liver cells in a damaged lobule are exposed to certain toxic effects following the infestation of larvae with resulting metabolic disturbances. It was clear from microscopic investigation that the grey-white spots on the liver observed on the third day consisted of intralobular focal infiltrations and interlobular septa thickened by cell infiltrations. The cell infiltration in this recent damage, was principally made up of histiocytes and relatively few eosinophil leucocytes. A marked change in the liver lesions had occurred by the tenth day. In the pigs killed after ten days, the haemorrha-

ges, liver cell necroses and the histochemical reactions in the surrounding liver cells had almost disappeared and were replaced by reparatory processes. This indicates that the acute liver damage reaches its climax when the *Ascaris* larvae penetrate into the liver parenchyma, and after that the processes of repair begin with the rapidity typical for the liver. The intralobular, but particularly the interlobular cell infiltrations had greatly increased. The cell infiltration had changed in character and was now completely dominated by eosinophil leucocytes. No remarkable increase in the connective tissue, however, was found at this stage. Thus, the grey-white areas principally consisted of infiltrations of eosinophil leucocytes and not of increased connective tissue, so the most commonly used term for these areas — parasite scar — is inappropriate at this stage.

The liver lesions underwent further changes up to the *63rd day*. Most of the infiltration of eosinophil leucocytes in the liver seemed to be of short duration, and to a surprisingly great extent had disappeared in the pigs which were allowed to live 63 days after beeing exposed to invasion of *T. cati*. At this stage only isolated, superficial areas remained, macroscopically similar to those observed earlier. Now however, the spots consisted of increased perilobular connective tissue with a moderate occurrence of eosinophil leucocytes. In the interior of the organ there was an area of another type, consisting of some follicle-like lymphocyte aggregations. From this it is clear, that most cell infiltrations appearing after an invasion of *T. cati* in the livers of swine are of fairly short duration and can disappear after a few months, leaving only a few spots in the form of connective tissue scars or focal lymphocytic infiltrations.

The parasitic lesions in the lungs were in general similar to those in the liver, but seemed to appear later in the lungs, probably because it takes longer for *T. cati* larvae to arrive there. On the 63rd day, the damage in the lungs had practically disappeared.

The number of eosinophil leucocytes in the blood was determined here only by differential counts of the blood smears, but the results should still reflect the variations in the content of eosinophil leucocytes in the blood at different times after the introduction of eggs. The relative content of eosinophil leucocytes in the blood seemed to run parallel with the content of eosinophils in the liver. On the third day, the relative number of eosinophil

leucocytes in the blood was, on the average, 3.5 %, thus somewhat raised; on the tenth day when the number of eosinophil leucocytes in the liver damage was at its greatest, the differential count was 15.6 % eosinophils, and on the 63rd day when the liver was almost back to normal the value was 2.5 %, approaching the 1.5 % of the control pigs.

It has thus been shown that liver lesions in svine, corresponding to those generally considered to be caused by *Ascaris suum*, can arise from migration of *T. cati*. As it is easy for pigs to pick up *T. cati* eggs from feed, litter and soil, it must be considered that parasitic liver lesions caused by *T. cati* constitutes a significant part of the spontaneously appearing cases. This may be one of the explanations why pigs are sometimes found with numerous parasitic spots in the liver, although they are free from *Ascaris suum* in the intestine.

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SUMMARY

In this paper, an investigation was made to determine whether liver lesions in swine could be caused by migration of *T. cati* larvae.

In the experiment, two pigs were given 35.000 *T. cati* eggs, and killed after three days, two pigs were given 20.000 eggs and killed after 10 days and four were given 100.000 eggs and of these, two were killed after 10 days and two after 63 days.

After only three days, a large number of small haemorrhages and grey-white spots on the surface and in the interior of the liver were observed macroscopically. Isolated *T. cati* larvae were found histologically in the intralobular liver tissue. Liver cell degeneration was found in the centre of the haemorrhages, glycogen was absent in the surrounding liver cells, and at the same time, the cytoplasm was silver positive. The grey-white spots proved to be principally composed of histiocytes with an admixture of eosinophil leucocytes. The liver damage seemed to be greatest on the third day.

On the tenth day, a morphological change in the lesions had occurred. The number of *T. cati* larvae was smaller. The haemorrhages and the degenerate liver cells had been resorbed. The grey-white spots, on the other hand, had increased somewhat in area and now consisted exclusively of interlobular infiltrations of eosinophil leucocytes. No noteworthy increase in the connective tissue existed at this stage.

On the 63rd day no *T. cati* larvae were found, the changes had largely disappeared and only isolated grey-white spots remained, consisting at this stage of new formations of interlobular connective tissue with a moderate number of eosinophil leucocytes. In one case the spot was composed of moderately well developed follicle-like lymphocyte aggregations.

It became clear that the grey-white spots were not necessarily chronic with a connective tissue proliferation, but could also consist of fresh, cellular infiltrations which could often be resorbed without leaving any trace.

The investigations show that invasion of *T. cati* in swine can give rise to parasitic liver lesions corresponding with those occurring spontaneously. This can be one of the explanations for the fact that swine are sometimes seen with an abundance of liver spots, but with no ascarids in the intestine.

It has also been shown that *T. cati* in swine causes visceral larva migrans of a type similar to that caused by *T. canis* and *T. cati* in man.

ZUSAMMENFASSUNG

Parasitärer Leberschaden beim Schwein, durch viscerale Larva migrans von Toxocara cati experimentell erzeugt.

Die vorliegenden Arbeit zielte auf die Untersuchung ab, ob Leberschäden beim Schwein durch wandernde *Toxocara cati*-Larven verursacht werden können.

In diesem Versuch wurden 2 Ferkeln zirka 35.000 *T. cati*-Eier verabfolgt, wonach die Tiere nach 3 Tagen getötet wurden; 2 Ferkel erhielten ungefähr 20.000 Eier und wurden nach 10 Tagen getötet, und 2 von 4 Ferkeln, die 100.000 Eier erhalten hatten, wurden nach 10 Tagen und die übrigen beiden nach 63 Tagen getötet.

Schon nach 3 Tagen liess sich makroskopisch eine grosse Anzahl kleiner Blutungen sowie grauweisser Flecken auf der Oberfläche der Leber und im Inneren derselben wahrnehmen. Durch histologische Untersuchung wurden vereinzelte *T. cati*-Larven im intralobulären Lebergewebe nachgewiesen. Im Zentrum der Blutungen lag Degeneration der Leberzellen vor, und in den benachbarten Leberzellen war das Glykogen verschwunden, wobei gleichzeitig das Zytoplasma silberpositiv reagierte. Die grauweissen Flecken bestanden hauptsächlich aus histiozytären Zellen mit geringgradiger Untermischung von eosinophilen Leukozyten. Der Leberschaden schien am 3. Tage seinen höchsten Grad zu besitzen.

Am 10. Tage war eine morphologische Veränderung der Schäden eingetreten. Die Anzahl der *T. cati*-Larven war geringer. Die Blutungen und die degenerierten Leberzellen waren resorbiert worden. Die grauweissen Flecken hatten dagegen an Umfang etwas zugenommen und bestanden nunmehr ausschliesslich aus interlobulären Infiltraten eosinophiler Leukozyten. Eine nennenswerte Bindegewebszunahme lag in diesem Stadium nicht vor.

Am 63. Tage wurden keine *T.-cati*-Larven ermittelt. Die Veränderungen waren in grossem Umfange verschwunden und nur vereinzelte grauweisse Flecken zurückgeblieben; in diesem Stadium stellten dieselben interlobuläre Bindegewebsneubildungen mit einer mässigen Menge eosinophiler Leukozyten dar. In einem Falle handelte es sich um einen interlobulären Herd, der aus lymphozytäre Zellen enthaltenden Follikeln bestand.

Wie es sich erwies, brauchten die grauweissen Flecken nicht immer chronischen Charakter mit Bindegewebe als Unterlage zu besitzen, sondern dieselben konnten auch aus frischen zelligen Infiltraten bestehen, die oft der Rückresorption anheimzufallen vermochten, ohne Spuren zu hinterlassen.

Die Untersuchungsergebnisse legen dar, dass eine Invasion von *T. cati* beim Schwein parasitäre Leberschäden bewirken kann, die mit den spontan auftretenden übereinstimmen. Dieses Verhalten kann als eine Erklärung dafür dienen, dass man zuweilen bei Schweinen Leberflecken wahrnehmen kann, während deren Darm frei von Ascariden ist.

Ferner wurde gezeigt, dass *T. cati* beim Schwein viszerale Larva migrans desselben Typs verursacht wie *T. canis* und *T. cati* beim Menschen.

SAMMANFATTNING

Parasitär leverskada hos svin, experimentellt framkallad genom visceral larva migrans av Toxocara cati.

I föreliggande arbete har undersökts huruvida leverskador på svin kunna orsakas av migration av *T. cati*-larver.

I försöket gavs 2 grisar ca 35.000 *T. cati*-ägg varefter grisarna avlivades efter 3 dagar, 2 grisar gavs ca 20.000 ägg varefter de avlivades efter 10 dagar och 4 gavs ca 100.000 ägg och av dessa avlivades 2 efter 10 och 2 efter 63 dagar.

Redan efter 3 dagar syntes makroskopiskt ett stort antal små blödningar samt gråvita fläckar på leverytan samt i det inre av levern. Histologiskt påvisades enstaka *T. cati*-larver i den intralobulära levervävnaden. I blödningarnas centrum förelåg levercellsdegeneration och i omgivande leverceller var glykogenet försvunnet och samtidigt blev cytoplasman silverpositiv. De gråvita fläckarna visade sig vara uppbyggda av huvudsakligen histiocytära celler med någon tillblandning av eosinofila leukocyter. Leverskadan tycktes vara som störst på 3:dje dagen.

På 10:de dagen hade en morfologisk förändring av skadorna skett. Antalet *T. cati*-larver var mindre. Blödningarna och de degenererade levercellerna hade resorberats. De gråvita fläckarna hade däremot ökat något i omfattning och bestodo nu uteslutande av interlobulära infiltrat av eosinofila leukocyter. Någon mämnvärd bindvävsökning förelåg ej i detta stadium.

På 63:dje dagen påvisades inga *T. cati*-larver. Förändringerna hade i stor utsträckning försvunnit och endast enstaka gråvita fläckar kvarstodo och i detta stadium bestodo de av interlobulära bindvävsnybildningar med måttlig mängd eosinofila leukocyter. I ett fall var fläcken uppbyggd av en interlobulär härd bestående av folliklar som innehöllo lymfocytära celler.

Det visade sig att de gråvita fläckarna ej alltid behövde ha kronisk karaktär med bindväv som underlag, utan de kunde även bestå av färska celliga infiltrat som ofta kunde återresorberas utan att lämna spår efter sig.

Untersökningarna visa att invasion av *T. cati* på svin kan ge parasitära leverskador som överensstämma med de spontant uppträdande. Detta förhållande kan vara en av förklaringarna till att man ibland ser svin, som ha rikligt med leverfläckar medan ascarider saknas i deras tarm.

Vidare har visats att *T. cati* på svin orsakar visceral larva migrans av samma typ som *T. canis* och *T. cati* orsaka hos människa.

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