PERSISTENT AND DISSEMINATED INFECTIONS WITH
STRONGYOLOIDES STERCORALIS IN IMMUNOSUPPRESSED DOGS

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Abstract—Grove D. I., Heenan P. J. and Northern C. 1983. Persistent and disseminated infections
with Strongyloides stercoralis in immunosuppressed dogs. International Journal for Parasitology 13:
483-490. The effects of immunosuppression on the course of infection in dogs infected with a human
strain of S. stercoralis were investigated. Four dogs were infected then 4 weeks later, three animals were
begun on prednisolone orally in a dose of 150 mg daily for 6 days each week. Rhabditiform larvae con-
tinued to be excreted in the stools. After 5 weeks of immunosuppression, one dog was autopsied; plentiful
parasites were found in the upper small bowel but worms were not seen in other tissues. Similar findings
were made in a second dog killed after 9 weeks of immunosuppression. In the third dog, the dose of
prednisolone was increased to 225 mg daily 15 weeks after infection then azathioprine 100 mg daily was
added 6 weeks later. This animal was killed 24 weeks after infection and evidence for multiplication of
S. stercoralis was obtained. Large numbers of adult worms, eggs and rhabditiform larvae were recovered
from duodenal fluid. Infective larvae were seen in homogenised lung, rhabditiform larvae were noted in
homogenised spleen and both rhabditiform larvae and adult worms were found in homogenised kidney.
Worms in all stages of development were seen in the urine. Histological examination revealed large
numbers of adult worms and enormous numbers of eggs and larvae in the duodenal mucosa. Worms
were seen throughout the length of the small intestine as well as in the colonic mucosa. The lungs dis-
played focal haemorrhages and larvae were seen in the alveolar spaces, bronchioles and bronchi.
Evidence of immunosuppression was provided by the gross atrophy of the thymus and the fall in anti-
Strongyloides antibody titres. In contrast to these dogs, larvae disappeared from the non-immuno-
suppressed dog by 13 weeks after infection. This animal was then immunosuppressed for 8 weeks but
parasites could not be found in either the stools or at autopsy. Two further dogs were immunosuppressed
before infection but they died soon thereafter. It is concluded that immunosuppression permits the per-
 sistence of infection with S. stercoralis and if continued for long enough and in sufficient degree, the
disseminated infection supervenes. This model may provide a means for assessing the efficacy of various
therapeutic regimens in overwhelming strongyloidiasis and for investigations of the host-parasite
relationship in this infection.

INDEX KEY WORDS: Strongyloides stercoralis; dogs; immunosuppression; hyperinfection; histology;
prednisolone; azathioprine.

INTRODUCTION

Disseminated strongyloidiasis has become increasingly recognised in humans in recent years
(Scowden, Schaffner & Stone, 1978; Ingra-Siegman, Kapila, Sen, Kaminski & Louria, 1981). This
condition, also known as overwhelming strongyloidiasis, or as massive infection or hyperinfection
with Strongyloides stercoralis, has been noted most frequently in patients who have been immuno-
suppressed, either as a result of disease such as lymphoma or protein–calorie malnutrition, or as a
consequence of the administration of immunosuppressive agents, usually corticosteroids.
Unfortunately, despite recognition of the condition and institution of anthelmintic therapy with
thiabendazole as well as antibiotics for secondary bacterial infections, perhaps half of these patients
still die. There is an urgent need, therefore, for the development of an animal model of this
syndrome, not only as an aid to the assessment of better therapeutic regimens, but also to provide a
system in which the underlying derangements in the host–parasite relationship can be investigated.

Such attempts have been hampered by the inability to produce patent infections with this worm in small
laboratory animals (Dawkins & Grove, 1982). We have recently shown that mongrel dogs can be
infected with a human strain of S. stercoralis (Grove & Northern, 1982). Some of these animals had
transient infections, while others developed a chronic, low-grade infection which presumably
reflected autoinfection. Thus, canine strongyloidiasis has strong resemblances to the infection
in humans. Consequently, we have attempted to
immunosuppress dogs infected with *S. stercoralis*, in order to determine whether disseminated strongyloidiasis would supervene.

**MATERIALS AND METHODS**

*S. stercoralis*. Worms were recovered originally from a human who had been infected while a prisoner-of-war in southeast Asia 35 years previously (Grove, 1980). This parasite has since been passaged in mongrel dogs; the methods of preparation of larvae, infection of animals and counting of larvae in the faeces have been described elsewhere (Grove & Northern, 1982).

**Dogs.** Adult male mongrel dogs ranging in weight from 15 to 22 kg were obtained from the local dog pound. The animals were washed, treated with the anthelmintics bumidine hydrochloride and pyrantel pamoate, then immunised with canine measles, parvovirus, distemper and hepatitis vaccines. The faeces were examined to ensure that they were free of helminths. Animals were housed separately and provided with food and water ad libitum.

**Prednisolone 25 mg tablets (Fawns and McAllan Pty. Ltd., Croydon, Victoria) and azathioprine 50 mg tablets (Wellcome Ltd., Concord, New South Wales) were administered orally directly by hand.**

**Antibodies.** Venous blood was collected and the serum examined for the presence of IgM and IgG anti-Strongyloides antibodies using an immunofluorescent technique described elsewhere (Grove & Northern, 1982).

**Autopsy.** Dogs were killed by intravenous injection of pentobarbitone. The macroscopic appearances of the organs were noted. Duodenal fluid and urine was aspirated. Specimens of lungs, hearts, stomach, duodenum, jejunum, distal ileum, colon, liver, spleen, kidney, bladder, pancreas, mesenteric lymph nodes, thymus, sternum and intercostal

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**Fig. 1.** Excretion of *S. stercoralis* larvae in the faeces at various times after infection. Individual dogs are numbered and dosage and duration of immunosuppressive treatment are indicated.
Strongyloidiasis in immunosuppressed dogs

RESULTS

In the first experiment, four dogs were infected with 5000 infective larvae of *S. stercoralis* p.o. Faeces were examined weekly and the numbers of larvae quantitated (Fig. 1). Patent infections were observed in all animals 3 weeks after infection. Four weeks after infection, 3 dogs were immunosuppressed by the administration of prednisolone 150 mg (approximately 8 mg/kg) orally for 6 days each week. Dogs were killed at various intervals.

**Dog 1**

The number of rhabditiform larvae in the faeces oscillated between 100 and 1000 per gram. Nine weeks after infection, the serum anti-Strongyloides IgM antibody titre was 1 : 16 and the IgG titre was 1 : 64. An autopsy was performed at this time; the dog weighed 17 kg. The thymus was atrophic but otherwise no macroscopic abnormalities were noted. Plentiful adult worms and rhabditiform larvae were found in the duodenal fluid. Worms were not seen in tracheal washings or in homogenised tissues.

Histological examination of the duodenum revealed plentiful adult worms, eggs and larvae. Adult worms were seen both free in the lumina of the crypts and in the superficial parts of the mucosa. There was a mild plasma cell infiltration of the villi, but this was also seen in normal dogs; there was no villous atrophy. The stomach, mid-small intestine, distal ileum and colon appeared normal and no worms were seen. No abnormalities were noted and no worms were seen in the liver, spleen, kidneys, pancreas, heart and skeletal muscle. The thymus was atrophic showing large sheets of adipose tissue with scattered small foci of lymphoid cells (Fig. 2).

**Dog 2**

The numbers of rhabditiform larvae in the faeces varied between 100 and 3000 per gram. Fourteen weeks after infection, the serum anti-Strongyloides IgM and IgG antibody titres were 1 : 4 and 1 : 256, respectively. An autopsy was performed at this time; the dog weighed 17 kg. The thymus was atrophic, but no other macroscopic abnormalities were noted. Worms were again recovered from the duodenal fluid but were not seen in homogenised tissues.

The histological findings were similar to those noted with the first dog except that *Giardia* trophozoites were seen on the duodenal mucosal surface.

**Dog 3**

Fifteen weeks after infection, the dose of prednisolone administered to this animal was increased to 225 mg (approximately 12 mg/kg daily). Six weeks later, azathioprine 100 mg (approximately 5 mg/kg daily) was added. The numbers of larvae in the faeces ranged from 100 to 10,000 per gram. Just before autopsy 24 weeks after infection, the larval count reached a maximum of 13,100 per gram faeces. At this time, the serum anti-Strongyloides IgM and IgG antibody titres were zero and 1 : 16 respectively. An autopsy was then performed; the dog weighed 13 kg.

The thymus could not be found, presumably due to complete atrophy, but no other gross abnormalities were present. In the duodenal fluid, frequent adult worms and large numbers of eggs and rhabditiform larvae were seen. In homogenised lungs, 11 infective larvae per gram of tissue were counted. In the spleen, there were 17 rhabditiform larvae per gram of tissue. In the kidneys, four adult worms (Fig. 3) and 21 rhabditiform larvae per gram of tissue were found. Adult worms, large numbers of rhabditiform larvae and a few second-, third- and fourth-stage larvae were seen in the urine.

Histological examination of the duodenum revealed large numbers of adult worms and enormous numbers of eggs and larvae (Figs. 4, 5). Adult worms were seen both within the mucosa and in the intestinal lumen. Eggs and larvae were found in mucosal vacuoles; no parasites were seen invading the submucosa or muscularis. There was a moderate mucosal infiltrate of both plasma cells and neutrophils. No villous atrophy was evident. Similar appearances were found in the terminal ileum with frequent adult worms and large numbers of embryos seen in the mucosa; there was no invasion of the submucosa or muscularis (Fig. 6). In the colon, large numbers of larvae were seen in the surface mucus and a few adult worms were seen in the mucosa (Fig. 7). The sinuses of the mesenteric lymph nodes were congested with histocytes. In the lungs, occasional larvae were seen in the alveolar spaces and in the bronchi and bronchioles (Fig. 8). There were intense pulmonary venous congestion and moderate focal alveolar haemorrhages but no significant inflammatory infiltration. The liver showed early fatty change but worms were not seen. The spleen, pancreas, heart and bladder were normal and no worms were seen. The kidneys showed proteinaceous deposits in some of the glomeruli, but despite examination of multiple sections, worms could not be found.

**Dog 4**

The fourth dog was infected at the same time as dogs 1–3 but was not immunosuppressed initially. Larvae were present in the faeces 3 weeks after infection, but were no longer detectable by 13 weeks. Fifteen weeks after infection, immunosuppression was begun with the administration of 150 mg prednisolone (approximately 8 mg/kg) daily in an attempt to light up an inapparent infection, should it be present. No larvae were seen in the faeces.
FIG. 2a. Thymus of a normal dog showing dense cellularity. (Scale bar 100 μm.)

FIG. 2b. Thymus of an immunosuppressed dog showing marked atrophy with fat replacement. (Scale bar 200 μm.)

FIG. 4. Low power view of duodenal mucosa showing large numbers of worms. (Scale bar 50 μm.)

FIG. 5. High power view of duodenum showing an adult worm in the mucosa. Note the paucity of inflammatory reaction. (Scale bar 20 μm.)
by direct microscopy or after culture during the ensuing 8 weeks. At the onset of immunosuppression, the serum anti-Strongyloides IgM and IgG antibody titres were 1 : 8 and 1 : 512, respectively. Twenty three weeks after infection, these titres were 1 : 4 and 1 : 512 respectively. An autopsy was then performed; the dog weighed 24 kg. No macroscopic lesions were seen apart from an atrophic thymus. Worms were not seen in duodenal fluid or in homogenised tissue. No significant abnormalities could be found on histological examination and worms were not seen.

**Dog 5**

In order to determine the effects of infection in an already immunosuppressed dog, a 9-week-old male mongrel pup weighing 3 kg was given 50 mg prednisolone and 50 mg azathioprine daily. Three days after beginning this administration, it was infected with 5000 infective larvae percutaneously. Two weeks after infection, rhabditiform larvae were present in the stool. During this period, however, it had developed diarrhoea and became lethargic and anorexic. Two weeks after infection the dog began to vomit and become markedly dehydrated. In view of the animal's discomfort, it was decided to terminate the experiment and an autopsy was performed 20 days after infection. At this time, IgM anti-Strongyloides antibody titres were present at a titre of 1 : 8 but IgG antibody was not detectable in the serum. Post-mortem examination disclosed large, bilateral, purulent pleural effusions and pneumatic consolidation of the lungs. The stomach was grossly distended with bile-stained fluid. The small intestine looked normal macroscopically. There was almost no faecal material in the large bowel. Large numbers of larvae were seen in the stomach contents and in duodenal fluid. No worms were seen in the urine, pleural fluid or homogenised tissues.

Histological examination of the duodenum revealed large numbers of adult worms and embryos as seen in other dogs. No worms were seen in other regions of the gastro-intestinal tract. The lungs demonstrated a patchy bronchopneumonia and purulent pleural exudate but larvae were not seen.

**Dog 6**

In view of the early demise of the previous dog, the latter experiment was repeated except that a male, mongrel dog 20 kg in weight was used. Immunosuppression was begun with prednisolone 200 mg (approximately 10 mg/kg) and azathioprine 100 mg (approximately 5 mg/kg daily). Three days later, it was infected with 6000 larvae p.c. A patent infection developed, but unfortunately, the dog died suddenly on a Saturday night three weeks after infection; no autopsy was possible.

**DISCUSSION**

Dogs are relatively resistant to immunosuppression with corticosteroids (Nara, Krakowka & Powers, 1979). However, considerable suppression of lymphocyte responsiveness to mitogens is produced when prednisolone is given in a dose of 1 mg/kg daily and further suppression of responsiveness occurs when prednisolone is given in a dose of 10
Fig. 6. High power view of distal ileum showing an adult worm in the mucosa. (Scale bar 20 μm.)
Fig. 7. High power view of colon showing an adult worm in mucosa. (Scale bar 20 μm.)
Fig. 8. High power view of bronchus showing a larva in the bronchial lumen. (Scale bar 20 μm.)
subsequent administration of corticosteroids did not

Meyers & Conner, 1974; Scowden Grille & Malaret, 1970; Powles, 1973; Purtilo, were confined to the upper small bowel. In dog 3, formation around worms. In dogs 1 and 2, worms disclosed adult worms and plentiful eggs and cytic or eosinophilic infiltration, nor any granuloma suppressed animals, there was no marked lympho-
larvae in the duodenal mueosa, as well as worms in the lumen. As might be expected in immune-

features noted in human studies (Civantos & Robinson, 1969; givera, Maldonado, Velez-Garcia, continued to be excreted in the stools and histological measurement of serum anti-Strongyloides antibody titres suggested impairment of specific antibody synthesis. Observations in non-immunosuppressed animals have shown that by 4 weeks after infection, the mean IgG anti-Strongyloides antibody titre is approximately 1 : 64 and that it is even greater (approximately 1 : 128) 20 weeks after infection (Grove & Northe,n, 1982). After initiation of immunosuppression, antibody levels declined, the IgG anti-Strongyloides antibody titre 24 weeks after infection in dog 3 being 1 : 16. Thirdly, the course of infection was altered in immunosuppressed dogs.

The pattern of larval excretion in the faeces of dog 4, which was not immunosuppressed, was similar to that usually observed (Grove & Northen, 1982). A few weeks after infection, parasites could no longer be detected in the stools. Furthermore, the subsequent administration of corticosteroids did not result in the appearance of larvae in the faeces which would have indicated the exacerbation of an inapparent infection. Finally, direct examination of the gut and other tissues at autopsy failed to disclose any evidence of continuing infection. In the three immunosuppressed animals, however, there was definite evidence of persistent infection. Larvae continued to be excreted in the stools and histological examination of the intestine confirmed the presence of adult worms and embryos in the duodenum. Since the intensity of infection, as indicated by faecal excretion of larvae, did not appear to be increasing as might have been expected if overwhelming infection was occurring, and as post-mortem examinations of dogs 1 and 2 failed to reveal evidence of disseminated infection, the dosage of corticosteroid given to dog 3 was increased. Six weeks later, there was still no increase in faecal larval excretion, so azathioprine was added to the immunosuppressive regimen. This was followed by an increase in faecal larval excretion to the maximal degree yet seen. Post-mortem examination of this animal demonstrated disseminated infection.

The pathological findings reproduced many of the features noted in human studies (Civantos & Robinson, 1969; Rivera, Maldonado, Velez-Garcia, Grillo & Malare,t, 1970; Powles, 1973; Purtilo, Meyers & Connor, 1974; Scowden et al., 1978). Histological examination of the small intestine of the dogs disclosed adult worms and plentiful eggs and larvae in the duodenal mucosa, as well as worms in the lumen. As might be expected in immunosuppressed animals, there was no marked lympho-
cytic or eosinophilic infiltration, nor any granuloma formation around worms. In dogs 1 and 2, worms were confined to the upper small bowel. In dog 3, which was most immunosuppressed, however, worms were found in the mucosa throughout the length of the small intestine as well as in the colon. Furthermore, in this dog, worms were found outside the gastrointestinal tract. Large numbers of larvae were seen in the lungs, both in the alveoli and in the large airways. Focal alveolar haemorrhages may have been due to damage by migrating larvae or may have reflected an azathioprine-induced thrombo-
cytopenia. Microscopical examination of whole worms in homogenised tissue demonstrated that the larvae were filariform, thus confirming that massive autoinfection was occurring. Larvae were also easily recoverable from the spleen and the kidneys. In these organs, however, they were rhabditiform stages, thus suggesting that they were produced locally. This was confirmed by the presence of adult worms in homogenised kidney. Worms were found in all stages of development in the bladder urine collected post-mortem. Urinary strongyloidiasis has been described in an otherwise normal human (Rifaat, Ghanam, Kanesky, Kholy, Hegazi & Ali, 1973); it may be more common in immunosuppressed patients than is generally realised.

A major problem in this study was the inordinate length of time it took to produce disseminated infection. Even though we did succeed in demonstrating dissemination, the severity of disease as indicated by gross pathological changes was not as great as has sometimes been noted in human patients. In an attempt to accelerate the onset of overwhelming strongyloidiasis, we then immuno-
suppressed two dogs before infection. Such immunosuppression, however, makes dogs susceptible to infection with a wide range of bacterial and other pathogens. Dog 5 developed marked vomiting and diarrhoea and became grossly dehydrated. The stomach was grossly distended with bile-stained fluid; this may have reflected pseudo-

 obstruction which has been described in stronylidiasis (Bartholomew, Butler, Bhasker & Jankey, 1977), but more probably was an ileus secondary to pneumonia and pleurisy. The pulmonary infection may have been the result of immunosuppression alone, the effects of migrating larvae in the lungs, or a combination of both. Similarly, dog 6 died suddenly, presumably from septicaemia. In both instances, sufficient time did not elapse for the development and widespread dissemination of a large number of worms.

Despite the difficulties with and limitations of this model, it is clear that infection persists and that auto-infection with dissemination will ultimately occur in immunosuppressed dogs. A system such as this is desirable for several reasons. It offers a means of assessing various therapeutic regimens in overwhelming strongyloidiasis. For example, it has been suggested on the basis of observation in mice, that thiabendazole is likely to prove a much superior agent than thiabendazole in the treatment of over-
whelming strongyloidiasis (Grove, 1982). Similarly, it has been shown that considerable resistance to infection with *S. ratti* in mice and rats can be transferred with immune serum (Dawkins & Grove, 1981; Murrell, 1981). Thus, both the effects of various drugs and the transfer of immune serum could be assessed in this model. Finally, the underlying mechanisms involved in strongyloidiasis remain enigmatic. How is it that *S. stercoralis* frequently evades the host’s defences successfully so that auto-infection occurs, but on the other hand, those same defences are usually able to contain and limit the intensity and spread of infection in immunocompetent animals? This system may provide a means for investigating these basic biological mechanisms.

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REFERENCES


