

FURTHER OBSERVATIONS ON PRENATAL HOOKWORM INFECTION OF DOGS¹

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The occurrence of prenatal infection with parasitic worms suggests a situation of considerable economic and public health importance. As far back as 1921, the possibilities of prenataally acquired helminthiases had been strongly enough indicated to warrant the appearance of general discussions of this subject in medical journals of both the United States (Cort, 1921) and France (Neveu-Lemaire, 1921). Although our information is still too limited to evaluate properly either the real importance of this phenomenon or the frequency and extent of its occurrence under natural conditions, there can be no doubt that it has been demonstrated more or less readily under experimental conditions in a number of host-parasite relationships. It is significant also to note that many of the recent contributions to our knowledge of this phenomenon were anticipated in the early discussions mentioned above.

It has been shown experimentally that puppies can become infected before birth with the round-worm, *Toxocara canis* (Fulleborn, 1921; Shillinger and Cram, 1923; Augustine, 1927), and with the hookworm, *Ancylostoma caninum* (Adler and Clark, 1922; Foster, 1932). On the basis of animal experimentation, it appears that intra-uterine infestation with the Japanese blood fluke, *Schistosoma japonicum* (Fujinami and Nakamura, 1911; Narabayashi, 1914, 1916), has also been demonstrated.

In addition to these, there are many helminths for which observational evidence has been presented to indicate the probability of prenataally acquired infestation by both human and animal hosts. In this group are the human hookworm, *A. duodenale* (Howard, 1917; DeLangen, 1923), the agent of hydatid disease, *Echinococcus granulosus* (Deve; 1918), the cattle round-worm, *Ascaris vitulorum* (Macfie, 1922; Griffiths, 1922), the hookworm of the pig, *Necator suillus* (Ackert and Payne, 1923), the sheep lung-worm, *Dictyocaulus filaria* (Neveu-Lemaire, 1912), and the dog heart-worm, *Dirofilaria immitis* (Shiplely, 1894; French, 1899; Hopkins, 1905). It is possible also that prenatal infection may occur with *Strongylus vulgaris*, since verminous aneurysms have several times been reported in foals which were too young to have developed such an advanced lesion after birth (Hutyra and Marek, 1926).

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Our present information suggests that the possibility of prenatal infection with any parasitic worm is conditioned upon (1) a blood-stream migration of the larvae, and (2) a tendency of the larvae to penetrate tissue barriers. It might be of interest to point out certain of the human helminths in addition to those discussed above which might be expected, on this basis, to be capable of infecting man prenatally. Among the nematodes, it appears that the above conditions are satisfied by *Ascaris lumbricoides*, *Necator americanus*, and *Strongyloides stercoralis*. It is not known that the larvae of *Trichinella spiralis* possess either the tendency or the ability to migrate from the blood stream, and it seems quite possible that they are transported to their final seat of encystment while still within the finer vessels. This may account for the apparently negative results of experiments which have tested for the possibility of intra-uterine infection with this form. Among the cestodes, other than *Echinococcus*, it is possible that prenatal infection might rarely occur in the case of *Taenia solium*. This possibility is limited to the development of the cysticercus stage which presumably would become established in a fetus only if an onchosphere traversed the placental barrier. Other than the schistosomes it appears that the only trematode of man with which prenatal infection might occur, is the oriental lung fluke, *Paragonimus westermani*. Since the larvae of *Paragonimus* migrate through the tissues rather than by way of the blood stream and are larger than larval schistosomes it is improbable, although perhaps not impossible, that prenatal infection would occur with this form.

The present study includes additional data on the occurrence of prenatal hookworm infestation in dogs. In an earlier report (Foster, 1932) evidence was presented to show that all of the puppies of two litters (numbering 5 and 8 pups, respectively) born to mothers infected with *A. caninum* larvae during pregnancy acquired prenatal infestations. It was shown that in these cases the development of the larvae in the prenatally infected puppies did not take place until after birth. The fact that more than ten per cent of the larvae given the mothers during gestation were recovered from the puppies as mature worms was taken as indicating a wide-spread migration of hookworm larvae after oral infection, a phenomenon which it was suggested may have been associated with the resistance of the hosts. The marked susceptibility of the puppies born to very resistant mothers was pointed out to be difficult to reconcile with a serological explanation of this type of resistance. These findings made it desirable to pursue further the studies of prenatal hookworm infection, particularly since no difficulties were being experienced in the breeding and rearing of dogs and since both specific and non-specific strains of the dog hookworm were available.

It was possible to obtain three litters of puppies from bitches which

were given only one dose of infective larvae during pregnancy and a fourth litter from a mother which was infected during late pregnancy with larvae of the "cat strain" of *A. caninum*.² The data from these experiments offer some interesting comparisons with the earlier results since in those cases reported previously the mothers were subjected to repeated infection during pregnancy.

The mother of the first litter had been given a total of 12,000 larvae *per os* over a period of 380 days, of which number, 5,000 were given in a single infection nine days before the puppies were born. She had been freed of worms from earlier infections, however, by anthelmintic treatment during pregnancy and the infective dose noted above was the only one given during this period. Two puppies were born and they were examined individually by the Lane Method on successive days, beginning seven days after birth. On the eleventh day one became positive to this examination, while the other did not. On the twelfth and thirteenth days the same condition prevailed and it was thought that only one of the puppies had acquired a prenatal infection. Since it was not necessary to autopsy these puppies in order to establish the fact that one of them was prenatally infected, they were kept available in case some additional experimental procedure should suggest itself. However, when these puppies were eighty days old, it was decided to autopsy the one which had become positive to Lane examination on the eleventh day. Only six hookworms were found. Finding this very light infestation made it desirable to obtain an autopsy record on the other pup of this litter, and in this case seven hookworms were recovered, one female and six males. It is not known whether this dog had been passing eggs immediately before autopsy but it seems reasonable to suppose that this infestation had been present since birth. Certain findings on the next litter indicate that the inability to recover eggs from this light infestation during the course of the early examinations might be explained by the possibility that the only female worm of the infestation was in copula during that time.

The second litter presented an interesting case, similar to that of litter 1, since in this instance also, a single infection given the mother produced very light infestations in the puppies. The mother of this litter was born in the laboratory March 30, 1931, and had successfully reared two litters previous to the one under discussion. This, her third litter, was born January 28, 1933. During the five months immediately pre-

² The so-called "cat-strain" of the dog hookworm, described by Scott (1929, 1929a, 1930) is a physiological variety of *Ancylostoma caninum*, the larvae of which are only slightly infective to dogs, but highly infective to cats. There is also a marked age resistance in the dog to infection with this strain, adult dogs being refractory to parasitism except under abnormal conditions, such, for example, as deficient diet (Foster and Cort, 1932; Foster and Daengsvang, 1932).

ceding this date, she had been given a total of 4,200 larvae in several doses but was freed of worms from these infections by anthelmintic treatment during pregnancy. It is apparent that at this age and under these conditions of infection she had become highly resistant to hookworms. A single infection of 2,000 larvae was given five days before the birth of 6 puppies. The mother developed a very light infestation showing an egg output of 25,000 to 50,000 eggs per day, which indicates a high degree of resistance although not a complete immunity. One of the puppies was killed and autopsied immediately after birth and a few hookworm larvae were recovered from the tissues with the use of the Baermann isolation apparatus. A second puppy was autopsied when nine days old and no worms were recovered, although an extended search was made. This does not necessarily mean that worms were not present, since at this age the developing worms were too large for recovery with the Baermann apparatus and too small to be seen easily with the unaided eye. Although a heavy infestation would not have escaped detection, it is fair to say that the finding of a 9-day-old larva, if only a few were present by examination of the macerated organs, would have been largely a chance discovery. Of the other four pups, three became positive to Lane examination on the eleventh and twelfth days. The fourth puppy continued to be negative when examined by the Lane method on successive days and was sacrificed on the sixteenth day. At autopsy, four worms were recovered (1 female, 3 males) and the absence of eggs in the stools may possibly be accounted for by the fact that the only female worm of the infestation was in copula. A fourth puppy of this litter was autopsied at 19 days of age and three worms were recovered (2 females, 1 male). It was considered unnecessary to sacrifice the remaining two members of the litter since the presence of worms was already known from the Lane examinations.

The third case was one in which a mature female dog, which had never before been infected, was bred and then given a single infection of 1,500 larvae thirty-two days before the birth of eight puppies. The interest in this case centered upon whether or not an infection given so early in pregnancy would result in a prenatally acquired infestation of the puppies. One of the puppies was autopsied when six days old and one each of ascaris and hookworm larvae were recovered from its tissues. The other seven were examined individually by the Lane method on successive days. Five of these became positive on the eleventh day and another one on the twelfth day. The infestations in these six puppies were undoubtedly light as indicated both by the Lane examinations and by the fact that the puppies were enjoying good health. These puppies were not autopsied as it was felt that the additional information to be obtained thereby would not justify the sacrificing of so valuable an ex-

perimental group. The eighth puppy was autopsied, however, on the thirteenth day and no worms were recovered.

The fourth case is the record of an infestation among a single litter of puppies which has been regarded, in many respects, as the most convincing as well as the most interesting experiment of this series. The mother was given *per os* a single dose of 9,000 larvae of the "cat strain" of *A. caninum* two days before the birth of five puppies. Although this dog had previously harbored hookworms, she was treated to negative in early pregnancy, after which she remained negative to Lane examination, and no infections were given except the one listed above. In addition to the fact that this strain of *A. caninum* is only slightly infective to dogs it has been found also to have a much longer prepatent period than is encountered with larvae of the straight dog strain. In this instance, the prepatent period of the "cat strain" in the mother was nineteen days, as she began passing eggs seventeen days after the puppies were born, showing a very light infestation for a while thereafter. Meanwhile, all five puppies had become positive to Lane examination when from fourteen to sixteen days of age. The prepatent period in the puppies was, therefore, shorter than that in the mother and every puppy had shown evidence of infestation with mature worms before the mother had begun to pass eggs. One of these puppies died when 56 days old, while the rest were killed and autopsied at various intervals between 41 and 73 days of age. The worm burdens were found to be uniform throughout the litter, varying from 19 to 27 worms per dog, with an average of 24 hookworms each.

DISCUSSION

On the basis of the observations described above, it seems reasonably clear that prenatal infestations with the dog hookworm, *A. caninum*, were established in the puppies of four litters. Up to the present, the author has studied the condition of infestation of 34 pups (including data from previous paper, *loc. cit.*), representing six litters, and 32 of these puppies were found infected with hookworms. The circumstances giving rise to such infestations were those of repeated or single exposure of resistant bitches to infective larvae during pregnancy. In previous experiments (Foster, 1932, pp. 114-115) it has been shown that the presence of maternal infestation during pregnancy and nursing is not a factor giving rise to prenatal infection. It appears, however, that exposure of the mother to infective larvae as early as 32 days or as late as 2 days before parturition (gestation normally 63 days) may result in intra-uterine infestation of the puppies.

Considerable significance has been attached to the fact that the puppies born to resistant mothers showed a marked susceptibility to infec-

tion. In his study of prenatal infestation with the dog *Ascaris* Augustine (*loc. cit.*) stated that of the two bitches infected during pregnancy, one showed no worms at autopsy, and the other only a few worms. The puppies born to these bitches, however, harbored heavy infestations. He looked upon this as indicative of an age immunity in the bitch against this parasite, the larvae showing a decided preference for fetal tissues. The same condition of maternal resistance prevailed in the studies of Shillinger and Cram (1923) and of Fulleborn (1929). The latter wrote in connection with the findings in his own case, that the intestine of the mother dogs, in consequence of "immunity," was usually not infected (1929, p. 23). It is significant that, in all these studies, there was a lack of resistance, even prenatally, in puppies born to very resistant mothers. On the assumption that the resistance of these bitches was effected by serum antibodies one would have anticipated a temporary protection of the puppies. On the other hand, if it be assumed that the maternal resistance was effected primarily by age, one would have expected a condition of susceptibility among the puppies. This observation has been regarded as lending some support to the view that the resistance encountered in these host-parasite relationships may not be of a serological nature.

The results suggest also that there may be a relationship between exposure to infection with the human hookworms during pregnancy and infant morbidity and mortality.

ABSTRACT SUMMARY

The administration *per os* of single doses of infective larvae of *A. caninum* to three pregnant bitches at intervals of 5, 9, and 32 days, respectively, before parturition resulted in prenatal hookworm infestations in all but one of twenty-one puppies. Although the mothers were very resistant to hookworms, the puppies did not appear to be protected on this account. One litter of five pups was prenatally infected with the "cat strain" of *A. caninum* as a result of administering larvae to the mother only two days before birth of the puppies. It is suggested that the occurrence of prenatal infection with parasite worms may be of economic and public health importance.

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