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Transmission of *Ancylostoma caninum* and *Alaria marcianae* in Coyotes (*Canis latrans*)

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ABSTRACT: One of seven female coyotes (*Canis latrans*) captured in Webb County, Texas during September 1986 and confined and mated in holding facilities at Millville, Utah whelped the following spring. The maternal female (>5-yr-old) and her five neonates were killed at 22 days postparturition. All were infected with adult *Ancylostoma caninum* and were passing eggs in their feces. Also, the neonates and maternal female were infected with immature and adult *Alaria marcianae*, respectively. These findings suggested that the transmammary route is an important transmission mechanism for acquisition of these species of helminths in coyotes. The lack of overdispersion in the frequency distribution of these parasites and infection of the entire litter indicated that transmission from the infected female was nonselective among the pups.

Key words: *Alaria marcianae*, *Ancylostoma caninum*, *Canis latrans*, coyote, experimental study, hookworm, intestinal diplostomid digenean, natural infection, transmammary transmission.

Pence and Windberg (1984) postulated that the transmammary route is the primary means for transmission of the hookworm *Ancylostoma caninum* and the diplostomid digenean *Alaria marcianae* in coyotes (*Canis latrans*) from southern Texas. This was based on very similar changes in population dynamics across variables of host age groups and seasons of these two otherwise very different species of helminths. Transmammary transmission is a common means for acquiring hookworm (Miller, 1971) and probably *A. marcianae* infections (Shoop and Corkum, 1987) in the domestic dog, a species phylogenetically close to coyotes (Bekoff, 1982). However, transmammary transmission of helminths in coyotes has not been demonstrated either experimentally or by examining naturally infected neonates. Because there are important differences in the helminth communities of domestic dogs and coyotes (Gier, 1968), and because hookworm infection has been proposed as a mortality factor in young coyotes (Gier, 1968; Pence and Windberg, 1984), it is desirable to determine the natural mechanisms of transmission of *A. caninum* in this host. The present study attempted to determine: (1) if naturally infected female coyotes transmit these helminths, particularly hookworm, to their offspring by the transmammary route; and (2) whether transmission is uniform or selective for individuals within litters.

Seven female coyotes were captured using leghold traps with tranquilizer tabs (Balser, 1965) in Webb County, Texas (27°54'N, 99°22'W). The females were held in 1.5- × 1.0- × 1.0-m cages for 2 to 12 days and fed jackrabbits (*Lepus californicus*) prior to transport to the U.S. Department of Agriculture facilities at Millville, Utah (Animal and Plant Health Inspection Service, Utah State University, Logan, Utah 84322, USA) on 25 September 1986. In Utah, these females were held initially in individual 1.3- × 4.0-m kennels for acclimation to captivity. In December, they were transferred to individual 0.1-ha enclosures surrounded by a 2.5-m chain-link fence. The enclosures were arranged in groups of three around an observation building which contained a den box for each enclosure. The coyotes were fed a quality controlled diet of a commercial fur-industry ration (Fur Breeders Agriculture Co-op, Logan, Utah 84321, USA) and given water ad libitum. All animals

were vaccinated against canine distemper, hepatitis, rabies, leptospirosis and parvovirus infections (Adenomune-7, Tech American Company, Omaha, Nebraska 68134, USA) on arrival at the Millville facility. Ages of coyotes were estimated by cementum annuli from microscopic sections of premolar teeth (Linhart and Knowlton, 1967). Human activity was restricted in the vicinity of the pens to reduce stress and facilitate conditioning the animals to captivity. At the time they were moved to the 0.1-ha pens, a captivity-conditioned male coyote was placed with each female and maintained with her throughout the normal breeding cycle. Subsequently, males were removed and females were subjected to minimal human disturbance during gestation.

After the normal whelping period, all pens were searched for litters. The only litter found consisted of five pups whelped by a >5-yr-old female. When the litter was 22-days-old, all coyotes (including the adult females) were killed with an injection of T-61 (Euthanasia Solution, American Hoechst Corporation, Sommerville, New Jersey 08876, USA). Examination of the uteri of the adult females revealed that the three youngest females (1- to 2-yr-old) had not implanted, three other females (3 to 5 yr old) had either resorbed or aborted implanted fetuses, and one >5-yr-old female had five placental scars.

The litter of the >5-yr-old female consisted of three females and two males. The small and large intestines of this adult and entire carcasses of the neonates were frozen at −4 C and shipped on dry ice to the Department of Pathology, Texas Tech University Health Sciences Center (Lubbock, Texas 79430, USA). All neonates were weighed in grams on a Mettler P1000 electronic balance (Mettler Instrument Corporation, Highstown, New Jersey 08520, USA) prior to necropsy. Tissue slices of approximately 2- to 4-mm thickness from the lungs of each neonate were fixed in 10% buffered formalin, processed and embedded in paraffin, sectioned at 4 to 6 μm and stained with hematoxylin and eosin. The collection of helminths from the small intestine was facilitated by sedimentation of the scraped mucosal contents in conical glasses. The residue was examined grossly and with the aid of a dissecting microscope. All helminths were removed, counted, sexed (hookworms) and classed as reproductively mature or immature. Nematodes were fixed briefly in glacial acetic acid, stored in a mixture of 70% ethanol with 10% glycerine by volume and examined in glycerine wet mounts following evaporation of the alcohol. Trematodes were fixed in a mixture of ethyl alcohol–formalin–acetic acid, stained in Semicohn's acetocarmine and mounted in Canada balsam. Representative specimens of *A. caninum* and *A. marcianae* were deposited in the U.S. National Parasite Collection (Agriculture Research Service, BARC-East, No. 1180, Biosystematic Parasitology Lab, Beltsville, Maryland 20705, USA; accession numbers 80161 to 80162).

The weights of the three female pups at 22 days of age were 554, 555 and 569 g; weights for the two males were 622 and 638 g. The three females had 25, 12 and 4 *A. marcianae* and 25, 17 and 11 *A. caninum*, respectively; males had 19 and 14 *A. marcianae* and 32 and 22 *A. caninum*. For all pups collectively, the mean (±standard error) number of hookworms and *A. marcianae* was 21.4 ± 3.6 and 14.8 ± 3.5, respectively. Within this small dataset, the frequency distribution for each species of helminth (4, 12, 14, 19 and 25 for *A. marcianae* and 11, 17, 22, 25 and 32 for *A. caninum*) appeared clustered about the mean with a single low and high value. The variance of each dataset was just significantly larger than the mean ($P < 0.05$) based on each frequency distribution and analyzed by chi-square values according to the methods of Bliss and Fisher (1953). However, the value of k, an inverse measure of overdispersion (Bliss and Fisher, 1953), was high for both species ($k = 4.68$ and 10.91 for *A. marcianae* and *A. caninum*, respectively). Thus, it appeared that

the distribution patterns of both species approached a normal frequency distribution across the neonate hosts.

The >5-yr-old female was infected with one adult specimen of *Toxascaris leonina*, one *Mesocestoides lineatus* strobila with mature proglottids, 103 mature *A. marcianae* and 18 adult *A. caninum*. Hookworm eggs in various developmental stages from undeveloped to containing a larva were found in direct fecal smears from the feces in the large intestine of all neonates and the female. Eggs of *A. marcianae* were observed in fecal smears from the female but not the pups.

All the hookworms from the five neonates were reproductively mature, except for two reproductively immature females in one individual. The ratio of male to female *A. caninum* was 1:1.5. Gross pathology in the small intestine of neonates was typical of low intensity hookworm infections, consisting of small areas of petechial hemorrhages on the mucosal surface located in the vicinity of the site of attachment for each hookworm. The intervening mucosa appeared normal and blood was not observed in the intestinal tract or in the fecal mass. There was no histological evidence of larval migration in the lungs.

All specimens of *A. marcianae* were reproductively immature. There was no evidence of a gross pathological response to this parasite in the small intestine and neither mesocercariae nor diplostomula were observed in tissue sections of the lungs of infected neonates. The normal body weights for their age (Gier, 1968; F. F. Knowlton, pers. obs.) and otherwise healthy appearance indicated that these infections had little effect on the pups.

The objectives of this study were not met entirely because of poor reproductive success among the coyotes. Young coyotes (1- to 2-yr-old) typically have low reproductive rates in southern Texas (L. A. Windberg, pers. obs.). Three of the seven females in this study were ≤2-yr-old. Although reproductive success in adult captive-reared coyotes approaches 90 to 95% (F. F. Knowlton, pers. obs.), reproductive success was appreciably less in our study which used females placed in captivity after they were mature.

Interpretations about helminth infections from one litter of coyote pups are tenuous. However, our data are consistent with previous studies in domestic dogs, particularly for hookworm infections (Miller, 1971). Although transmammary transmission of hookworm and *A. marcianae* can be demonstrated conclusively only by identification of third stage larvae and mesocercariae, respectively, in milk of lactating females, our results provide indirect evidence for transmammary transmission of these infections that is consistent with other studies (Pence and Windberg, 1984; Shoop and Corkum, 1987), especially for *A. marcianae*. Transmammary transmission was the most likely route for infection with *A. marcianae* in these neonates because milk was the only source of nutrition from birth to 22 days of age. Transmammary transmission of *A. marcianae* was suggested, but not demonstrated in the domestic dog (Shoop and Corkum, 1987). There was minimal chance that neonates were exposed to *A. caninum* from an external source because the den box had a concrete bottom lined with dry wood shavings. Almost all the specimens of *A. caninum* found in neonates were reproductively mature. Because *A. caninum* requires at least 15 to 18 days to reach reproductive maturity after initial infection (Levine, 1980), the neonates were infected apparently as fetuses or within the first week of life when they were in the den box.

The normal frequency distribution of helminths observed across the entire litter suggested a uniform and consistent pattern of transmission. This supports the contention that the overdispersed distribution of hookworms and *A. marcianae* in 6- to 8-mo-old juvenile coyotes from wild populations (Pence and Windberg, 1984) resulted from differences among litters rath-

er than within individual litters. In domestic dogs, fatal neonatal ancylostomosis most often occurs in a female's first litter, with the dose of hookworm larvae from mother to neonates successively reduced in subsequent litters (Miller, 1971). The hookworm larvae which are transmitted from the female to her neonates are sequestered as dormant larvae and are transmitted from females to the pups at each whelping. Apparently, a similar mechanism exists for *A. marcianae* in experimentally infected cats (Shoop and Corkum, 1987). This may explain the low intensities of hookworms and *A. marcianae* in the litter whelped and reared by the >5-yr-old female coyote in our study.

The potential mortality resulting from hookworm infection may selectively predispose initial litters of younger female coyotes to greater losses than litters of older females. This could be a mechanism impacting the dynamics of the coyote population in southern Texas.

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