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EXPERIMENTAL SALMON POISONING DISEASE IN JUVENILE COYOTES (*CANIS LATRANS*)

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Abstract: Salmon poisoning disease (SPD) was experimentally induced in juvenile coyotes (*Canis latrans*). The disease was lethal in 11 of 12 coyotes within 15 days after inoculation with 1,000 or 4,000 metacercariae of *Nanophyetus salmincola*. Clinical manifestations of the disease included lymph node enlargement, anorexia, pyrexia, diarrhea and death. Coccoid bodies indistinguishable from rickettsiae were observed in macrophages of spleen, liver, lymph nodes, and duodenum. Percentage recovery of adult trematodes from metacercariae administered was 23% from 12 inoculated coyotes, compared to 13% in one inoculated dog. Juvenile coyotes appear to be highly susceptible to experimental SPD.

INTRODUCTION

Salmon poisoning disease (SPD) is a rickettsial disease which primarily affects canids in the Pacific Northwest (Knapp and Millemann, 1970; Millemann and Knapp, 1970; Schlegel et al., 1968). Two species of rickettsiae, *Neorickettsia helminthoeca* and Elokomin Fluke Fever agent, alone or in combination, are the etiologic agents of this disease (Cordy and Gorham, 1950; Philip et al., 1954). These rickettsiae are carried by the fluke *Nanophyetus salmincola* throughout all stages in the life cycle and infect canids when metacercariae containing rickettsiae are eaten in fish. Salmon poisoning disease is highly pathogenic in the domestic dog (*Canis familiaris*) and has been studied extensively (Bosman et al., 1970; Cordy and Gorham, 1950; Farrell et al., 1955; Simms et al., 1932). Clinical manifestations of the disease in the domestic dog include pyrexia, anorexia, lymph node enlargement and death. Diarrhea, vomition and conjunctivitis are also observed frequently (Cordy and Gorham, 1950). The coyote (*Canis*

latrans) is reported to be susceptible to SPD (Cram, 1926; Donham and Simms, 1927; Schlegel et al., 1968). This study further characterizes SPD in juvenile coyotes.

MATERIALS AND METHODS

Seventeen coyotes (*Canis latrans*) approximately 8 weeks of age were divided randomly into groups of two or three and maintained in standard dog kennels with concrete floors. Coyotes were originally obtained from dens in an area of Whitman County, Washington, where SPD has not been identified. Each animal was vaccinated with 1 ml of a killed mink enteritis vaccine[□] subcutaneously. They were acclimatized in the kennels for 7 days before inoculation with metacercariae. Two domestic crossbred dogs (approximately 5 mo of age) were also maintained in separate kennels in the same room and vaccinated on the same day as the coyotes. On the day of inoculation, blood was collected via jugular puncture and a differential white cell count was determined. A sec-

□ Biovac ADF, United Animal Science Division, Middleton, Wisconsin 53562, USA.

ond blood sample was collected when animals were clinically ill, and similar hematology data were obtained.

Approximately 500 coho salmon (*Oncorhynchus kisutch*), steelhead salmon (*Salmo gairdneri*) and cutthroat trout (*Salmo clarki*) were obtained from the Elokomin Salmon Hatchery and Beaver Creek Hatchery in Cathlamet, Washington. Both hatcheries are in an enzootic area of SPD. Fish were examined for metacercariae of *N. salmincola* by removing the kidney and heart from each of 50 fish and preparing compressed tissue wet mounts for microscopic examination. Metacercariae were counted at 40X using a dissecting microscope. Kidneys and hearts from all fish were macerated with a scissors and the resulting mixture was mixed with distilled water. Ten 1 cc aliquots were withdrawn and examined and the total number of metacercariae was determined.

Six coyotes (two pens) and one domestic dog were each administered 4,000 metacercariae per os with a syringe. Six coyotes (two pens) were each administered 1,000 metacercariae per os. Five coyotes (two pens) and one domestic dog were not administered metacercariae but were maintained as controls in the same room as the inoculated animals. All animals were fed a commercial dry dog food ration and provided water *ad libitum*.

Daily physical examinations and rectal temperatures were taken. Body weight was measured every other day. Feces were collected on alternate days and examined by fecal flotation (sugar soln., sp. gr. 1.27) for the presence of parasite eggs.

Postmortem examinations were performed when animals died or at the termination of the experiment, 15 days after inoculation. Sections of lymph nodes, spleen, liver, duodenum and jejunum were stained with hematoxylin and eosin or with Giemsa. Parasites in

the intestinal contents were identified and counted.

Specimens of *N. salmincola* from coyotes were deposited in the U.S. National Parasite Collection, Beltsville, Maryland, and assigned accession No. 76460.

RESULTS

All three species of fish were found to be infected with 0-131 (\bar{x} =78) metacercariae of *N. salmincola* per fish.

All 12 inoculated coyotes had clinical signs of SPD and 11 of 12 died within 15 days (\bar{x} =10.7) after inoculation (Table 1). The surviving inoculated coyote was moribund and was euthanized on post inoculation day 15. Signs associated with SPD in coyotes were enlarged peripheral and internal lymph nodes, enlarged and hyperemic tonsils, diarrhea, fever, anorexia, weight loss and death.

Eggs of *N. salmincola* were observed in feces of all inoculated coyotes between 4 and 8 (\bar{x} =6.2) days post inoculation. The number of adult *N. salmincola* recovered was 9 - 2,617 (\bar{x} =583) which represented a 23% recovery from metacercariae administered (Table 1). None of the uninoculated control coyotes became sick or died, and flukes were not recovered at necropsy.

Average weight of all coyotes on the day of inoculation was 2.20 kg. Weight loss during the 15 day experiment averaged 319 g in the inoculated coyotes, compared to an average weight loss of 34 g in the uninoculated controls.

Enlarged cervical and prescapular lymph nodes were palpated as early as 3 days after inoculation and persisted during the experiment. Diarrhea, fever, anorexia and weight loss were usually observed after day 4. Body temperatures above 40 C were usually measured 6 and 7 days after inoculation and dropped to 35-37 C before death. Differential white cell counts from inoculated coyotes

TABLE 1. Summary of results from coyotes and dogs inoculated with metacercariae of *Nanophyetus salmincola*.

| Group | Mean Day of Death (Range) | Mean Number of Flukes Recovered at Necropsy (Range) |
|--|---------------------------|---|
| Uninoculated coyotes (N=5) | 15 ^a | 0 |
| Uninoculated dog (N=1) | 15 ^a | 0 |
| Inoculated coyotes 1,000 metacercariae (N=6) | 11 (11-14) ^b | 124 (9-196) ^c |
| Inoculated coyotes 4,000 metacercariae (N=6) | 10 (9-10) | 1,042 (314-2,617) |
| Inoculated dog 4,000 metacercariae (N=1) | 15 ^a | 506 |

^aKilled on day 15.

^bOne coyote was killed on day 15.

^cMany disintegrating flukes were also present in the coyote with 9 flukes, but were not counted.

before death showed an 89% neutrophilia; the remaining cells were lymphocytes.

Histologically, the most severe changes were confined to lymphoid tissue. Mesenteric lymph nodes were enlarged, and were characterized by loss of discrete lymphoid follicles and mature lymphocytes, an abundance of macrophages, and focal necrosis. Necrotic foci were present in up to 30% of some sectional areas. Macrophages typically contained numerous cytoplasmic coccoid bodies up to 0.5 μ m in diameter. These bodies were dark blue in Giemsa-stained sections. Occasional macrophages also contained phagocytosed erythrocytes. Duodenal and jejunal microvilli were widened and blunted and had necrotic tips. The lamina propria of such villi frequently were filled with plasma cells and macrophages. Typical Giemsa-positive

coccoid bodies were seen in the cytoplasm of these macrophages.

The single inoculated dog had clinical signs typical of SPD and was moribund, but did not die within 15 days post inoculation. Lesions of SPD were present histologically, and 506 flukes were recovered. The uninoculated control dog was unaffected; flukes were not recovered at necropsy.

DISCUSSION

Results of this experiment confirm the lethal effect of SPD in juvenile coyotes and corroborate results of others who experimentally infected coyotes with SPD (Donham and Simms, 1927). Lesions were comparable to those of natural and experimental SPD in domestic dogs (Cordy and Gorham, 1950).

Although SPD complex may be caused by one or more rickettsiae, it is likely that

the effect is lethal to a majority of coyotes. In this experiment, we did not specifically identify the rickettsiae, but from previous work we assume that the agent was *N. helminthoeca* and/or Elokomin Fluke Fever agent.

Fish are not important items in the diet of free-ranging coyotes (Grimm, 1940;

Springer, 1980), but we have observed that coyotes in captivity will eat large quantities of fish. The role of SPD in wild coyote populations in the Pacific Northwest is unknown, but one might speculate that the disease may affect local population dynamics, especially in areas where infected fish are accessible.

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