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Source: Journal of Wildlife Diseases, 23(3) : 412-417

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-23.3.412>

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SALMON POISONING DISEASE IN JUVENILE COYOTES: CLINICAL EVALUATION AND INFECTIVITY OF METACERCARIAE AND RICKETTSIAE

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ABSTRACT: Clinical salmon poisoning disease (SPD), and survival of *Neorickettsia helminthoeca* and metacercariae of *Nanophyetus salmincola* in fish were evaluated experimentally in 12-wk-old coyotes (*Canis latrans*) to determine the potential of SPD for biological control of coyotes. Coyotes readily ate fish that contained metacercariae and rickettsiae. They developed diarrhea, anorexia and lethargy within 7 days after feeding. Infected coyotes lost 58% of their body weight when compared to uninfected controls. They died or became moribund and were euthanized within 17 days after feeding. Rickettsiae were present in the macrophages of lymph nodes of all affected coyotes. Clinical disease occurred in coyotes fed fresh fish, but not in coyotes fed fish stored at 4 C for ≥ 30 days or at -20 C for 14 days. Metacercariae in fish were viable after 60 days at 4 C. These trematodes developed in coyotes, but clinical SPD did not occur. This indicated survival of metacercariae, but not rickettsiae. Metacercariae were not viable after 14 days at -20 C.

Key words: Salmon poisoning diseases, coyotes, *Nanophyetus salmincola*, *Neorickettsia helminthoeca*, experimental infections.

INTRODUCTION

Salmon poisoning disease (SPD) is a fatal disease in coyotes (*Canis latrans*) and is caused by *Neorickettsia helminthoeca* (Millemann and Knapp, 1970; Knapp and Millemann, 1981; Foreyt et al., 1982; Gorham and Foreyt, 1984). Naturally occurring SPD is ecologically limited to coastal areas of Washington, Oregon and northern California where the intermediate snail, *Oxtrrema silicula* occurs (Knapp and Millemann, 1981; Wilson and Foreyt, 1985), but SPD can occur in distant locations where infected fish have been transported and fed to domestic or wild canids (Foreyt, unpubl.).

The purpose of this study was to evaluate the potential of SPD as a biological control agent of coyotes. Survival of metacercariae and rickettsiae at different temperatures was evaluated to determine the effects of cold storage on disease transmission. Results of the study are readily

applicable to the epidemiology of SPD in all susceptible canine species.

MATERIALS AND METHODS

Twenty coyote pups (10 females and 10 males) <14 days of age were taken from two dens in Whitman County, Washington in April 1985. Pups were bottle fed a milk formula (385 ml evaporated milk, 385 ml water, 10 ml of corn syrup, and 2 whole eggs mixed in a blender) four times a day until weaning at 5 wk of age. Pups were maintained indoors at the Laboratory Animal Research Center, Washington State University (WSU). Weaned coyotes were maintained in pairs indoors in standard dog runs (2.5 \times 4.0 m) and fed a standard commercial dry dog ration (Purina Puppy Chow, Ralston Purina Company, St. Louis, Missouri 63164, USA) or the dry diet softened with goat's milk. Food and fresh water were available at all times.

Chinook salmon (*Oncorhynchus tshawytscha*) approximately 9.0-11.5 (\bar{x} = 10.5) cm long and weighing 7.6-12.6 (\bar{x} = 9.4) gm were obtained from the McAllister Hatchery near Olympia, Washington and transported on ice to WSU. Previous findings indicated that all fish

TABLE 1. Results of feeding approximately 100,000 metacercariae of *Nanophyetus salmincola* to coyotes.

Group	Number of coyotes	Mean day of death postinfection (range)	Trematode eggs present on day 14	Rickettsiae present at necropsy	Mean number trematodes recovered (range)
(I) Fed fresh fish	8*	16 (14–17)	positive	positive	196,192 (77,479–248,912)
(II) Fed 30-day-old fish (4 C)	2	no death	positive	negative	23,394 (16,348–30,440)
(III) Fed 60-day-old fish (4 C)	2	no death	positive	negative	218 (0–436)
(IV) Fed 90-day-old fish (4 C)	2	no death	negative	negative	0
(V) Fed 14-day-old fish (–20 C)	2	no death	negative	negative	0
(VI) Controls	4	no death	negative	negative	0

* Two coyotes were treated with tetracycline on day 12 and did not die of SPD; euthanatized on day 30. No rickettsiae were observed in lymph nodes.

from this hatchery are infected with large numbers of metacercariae of *Nanophyetus salmincola*, and SPD is a common disease in domestic dogs in that area. Kidneys were removed from 10 salmon chosen randomly and the entire kidney from each was pressed between two glass plates (8 × 8 cm). Metacercariae per kidney were counted using 15× magnification. The average number of metacercariae per kidney was 973 ± 84 (SD). Based on our unpublished observations, approximately one-half of all metacercariae are in the kidney. Therefore, we estimated each fish contained 1,946 metacercariae. Fish were stored in groups of 50 at 4 C or –20 C until they were fed to coyotes (Table 1).

Each of eight coyotes (two per kennel) received 51 fresh fish to provide an average intake of 100,000 metacercariae. In addition, two coyotes were each fed 51 fish stored for 30 days at 4 C, for 60 days at 4 C, for 90 days at 4 C, or 14 days at –20 C. Four control coyotes that were not fed fish were maintained throughout the experiment in the same room with coyotes that received fish. Coyotes were observed daily for changes in feed intake and in characteristics of the feces.

Feces were collected from each coyote on the day they were fed fish and 14 days later, and from control coyotes at the same time. Feces were examined for parasite eggs (primarily *N. salmincola*) using a sugar flotation technique (sp. gr. = 1.27). All coyotes were weighed 14 days after fish consumption.

Blood samples were collected from the jugular vein from all coyotes 12 days after fish consumption, and from control coyotes at the same time. Total and differential leukocyte

counts were performed on fresh blood and smears stained with a Wright's stain. Sera were separated from clotted blood samples within 1 hr after collection, and refrigerated (4 C). Serum samples were analyzed for blood urea nitrogen (BUN), creatinine, calcium, inorganic phosphorus, albumin, alkaline phosphatase (SAP), alanine transaminase (ALT), glucose and cholesterol. Concentrations were measured by automated clinical procedures (Gemsac Electro-Nucleonics, Fairfield, New Jersey 07022, USA). Data between groups were compared by the Student's *t*-test. Statistical significance was determined at $P \leq 0.05$.

Coyotes that died or were moribund and euthanatized were submitted to the Washington Animal Disease Diagnostic Laboratory for necropsy. The remaining coyotes were euthanatized 30 days after fish consumption, and necropsied. Representative tissues from each animal were fixed in 10% buffered formalin and processed routinely for histology evaluation. Sections of enlarged lymph nodes were stained with Giemsa stain and examined for rickettsiae. The small intestines were opened, small samples were fixed in formalin, and the remainder was flushed with water and the mucosa was scraped. Contents and scrapings were examined using 10× magnification and all trematodes were counted.

Specimens of *N. salmincola* were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, USA (No. 70112).

RESULTS

Coyotes readily ate all fish within 8 hr after they were placed in the kennels.

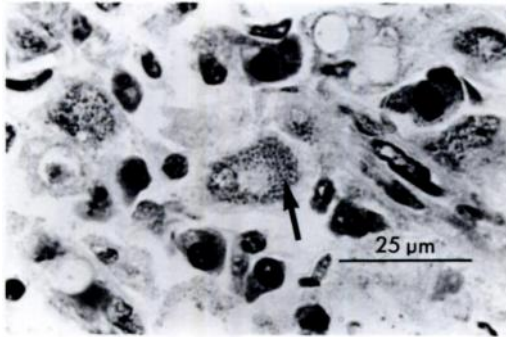


FIGURE 1. Rickettsiae of *Neorickettsiae helminthoeca* in periphery of a macrophage (arrow) of a mesenteric lymph node of coyote with salmon poisoning disease. Giemsa stain.

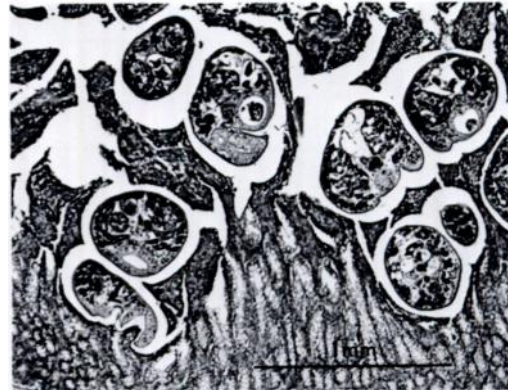


FIGURE 2. *Nanophyetus salmincola* in the mucosa of the small intestine of infected coyote. H&E stain.

Trematode eggs were not detected in coyote feces on the day of exposure. Numbers of trematodes recovered, occurrence of eggs in feces, and presence of rickettsiae in lymph nodes are summarized in Table 1. Control coyotes did not develop clinical signs of SPD and trematode eggs were not detected in their feces.

Adult trematodes were recovered at necropsy from all coyotes fed fresh fish and fish stored for 30 days at 4 C (Table 1). The average number of trematodes recovered from coyotes fed fresh fish was 196,192, which was higher than the estimated number of metacercariae given (100,000). Average numbers of trematodes recovered from coyotes fed fish stored at 4 C for 30, 60 or 90 days were 23,394, 218, and 0, respectively. Trematodes were not recovered from the coyotes fed fish stored at -20 C for 14 days, or from control coyotes (Table 1).

All eight coyotes fed fresh fish had clinical signs consistent with SPD. By the seventh day postexposure, coyotes were diarrheic, anorectic and lethargic. During the initial 14 days after ingesting fish, infected coyotes lost approximately 58% of their body weight (\bar{x} = 2.14 kg, range = 1.90–2.77 kg) when compared to controls (\bar{x} = 5.07 kg, range = 4.32–6.14 kg). Six of the eight coyotes died or were moribund and

were euthanatized on days 14–17 (Table 1). Rickettsiae were observed in lymph nodes of all six coyotes that died (Fig. 1). Two clinically affected coyotes were treated on the twelfth and thirteenth day postinfection with tetracycline at approximately 8 mg/kg body weight intravenously BID. Both subsequently recovered.

At necropsy, all coyotes were emaciated. They were without subcutaneous, mesenteric, omental, epicardial or perirenal fat. Mesenteric lymph nodes were markedly enlarged (to 3 × 1 × 1 cm), pale grey to yellow, and firm. A few *Toxascaris leonina* and *Uncinaria stenocephala* were recovered from the small intestines of three coyotes.

Edema, histiocytosis and focal necrosis characterized mesenteric and cervical lymph node sections. Lymph node macrophages were large and pale, with peripheral nuclei, and contained Giemsa-positive coccoid organisms less than 1 μm in diameter (rickettsiae). Numbers of affected macrophages, and numbers of organisms per cell were small. Each section of small intestine from the eight coyotes contained trematodes (Fig. 2). Mucosal changes were limited to areas of lamina propria that contained trematode eggs, with associated accumulations of macro-

TABLE 2. Hematologic and serum biochemical values of control coyotes and coyotes affected with salmon poisoning disease.

	Controls (n = 4)		Infected (n = 5)	
	Mean ± SD	Range	Mean ± SD	Range
Leukocyte count (cells/μl)	7,425 ± 1,284	5,900–8,800	6,825 ± 1,485	5,900–9,200
Hemoglobin (g/dl)	11.5 ± 0.8	10.5–12.4	11.0 ± 1.4	9.9–13.1
Packed cell volume (%)	34.5 ± 2.5	32–38	32.8 ± 3.1	28–40
Plasma total protein (g/dl)	5.2 ± 1.7	4.5–6.5	4.4 ± 0.6	3.9–5.3
Leukocyte differential				
Segmented neutrophils				
%	69 ± 10	55–78	81 ± 18	55–94
Cells/ml	5,084 ± 933	4,071–6,318	5,521 ± 2,470	2,992–8,004
Lymphocytes				
%	17 ± 11	3–30	15 ± 26	6–38
Cells/ml	1,282 ± 1,000	243–2,640	987 ± 1,122	272–2,660
Monocytes				
%	2.3 ± 1.3	1–4	2 ± 1	0–3
Cells/ml	175 ± 118	69–352	139 ± 127	0–276
Eosinophils				
%	12 ± 4	7–17	0.8 ± 1.0	0–2
Cells/ml	884 ± 304	483–1,377	58 ± 61	0–140*
Band cells				
%	0	0	1.8 ± 1.3	0–3
Cells/ml	0	0	121 ± 134	0–276 ^b
BUN (mg/dl)	21.3 ± 5.1	17–27	52.2 ± 31.3	20–99
Creatinine (mg/dl)	0.6 ± 0.1	0.5–0.7	0.3 ± 0.2	0.1–0.5*
Calcium (mg/dl)	10.8 ± 0.6	10.2–11.5	7.6 ± 1.24	6.5–9.7 ^b
Inorganic phosphorus (mg/dl)	8.5 ± 0.7	7.8–9.2	5.4 ± 1.2	4.3–7.4 ^b
Albumin (g/dl)	2.5 ± 0.2	2.3–2.7	2.0 ± 0.2	1.7–2.3 ^b
Alkaline phosphatase (IU/L)	318 ± 45.3	276–379	79.4 ± 66.7	27–169 ^b
Alkaline transaminase (IU/L)	21.9 ± 4.7	16.2–27.6	44.0 ± 36.5	13.8–102.3
Glucose (mg/dl)	110.5 ± 19.9	83–127	50.6 ± 32.7	16–92 ^b
Cholesterol (mg/dl)	134.5 ± 19.8	107–150	85.2 ± 63.3	42–196

* Significantly different from controls ($P < 0.05$).

^b Significantly different from controls ($P < 0.01$).

BUN = blood urea nitrogen; SAP = serum alkaline phosphatase; ALT = alanine transaminase.

phages and plasma cells. Macrophages, lymphocytes and plasma cells were commonly deposited in meninges and perivascular spaces of cerebral cortex and cerebral white matter of the brain. Other organs examined were considered normal.

Coyotes fed fish stored for 30 or 60 days at 4 C developed transient diarrhea from days 8–30, but did not have enlarged lymph nodes and all survived. All had trematode eggs present in feces 14 days after consumption of fish and adult trematodes were recovered from three of four coyotes 30 days after consumption of infected fish (Table 1). Control coyotes and coyotes fed

fish stored for 90 days at 4 C or 14 days at –20 C did not become clinically ill and eggs were not detected in feces. Trematodes were not recovered from the intestines, and rickettsiae were not observed histologically.

Hematologic and selected serum biochemical values from control coyotes and coyotes fed fresh fish 12 days previously are summarized in Table 2. Infected coyotes at this time were diarrheic, lethargic, anorectic, and had enlarged lymph nodes. Infected coyotes had higher numbers ($P < 0.01$) of band cells (unsegmented leukocytes), lower numbers ($P < 0.05$) of eosin-

ophils and lower concentrations ($P < 0.01$) of creatinine, glucose, calcium, inorganic phosphorus, albumin and alkaline phosphatase than control coyotes (Table 2).

DISCUSSION

Obviously, we underestimated the number of metacercariae present in fish based on the number of trematodes recovered at necropsy of the coyotes. Since coyotes were fed in pairs, it is likely that one coyote in each kennel ate more metacercariae than the other. However, the consistently high numbers of trematodes recovered indicated that the fish kidneys contained $\leq 25\%$ of the total metacercariae per fish, rather than the 50% we estimated initially.

Coyotes stopped eating about 6 days after fish consumption and we concluded the lack of energy intake directly resulted in significant loss of weight when compared with controls. Significant decreases in serum creatinine, calcium, inorganic phosphorus, albumin, alkaline phosphatase and glucose in infected coyotes were attributed to lack of energy intake. The increase in band cells was probably due to the infection. The decrease in eosinophils was probably due to the stress of infection.

Rectal temperatures of coyotes were taken during the experiment, but we did not include the data because of the wide variation between coyotes and from day to day. The longer a coyote was restrained, the higher the recorded temperature. Therefore, we concluded this measurement to be unreliable unless coyotes were acclimated to frequent handling.

These experiments confirmed the lethality of SPD in juvenile coyotes and support results reported previously in coyotes and dogs (Simms et al., 1931; Cordy and Gorham, 1950; Philip et al., 1954; Foreyt et al., 1982). Although metacercariae will remain viable at least 60 days at 4 C, the rickettsiae apparently do not survive for 30 days at 4 C. Metacercariae did not survive storage for 90 days at 4 C or 14 days

at -20 C, and clinical disease did not occur in coyotes fed these fish. Rickettsiae have been reportedly maintained at -20 C in lymph nodes (Philip, 1954), but our findings indicate that if rickettsiae were viable in frozen fish, disease did not result after oral feeding. It is possible that rickettsiae do not survive freezing in fish, or that a live trematode may be necessary for the transfer of rickettsiae into the bloodstream under natural conditions.

Domestic dogs affected by SPD are treated routinely with tetracycline (Gorham and Foreyt, 1984). As indicated in our experiments, a similar treatment is effective in coyotes.

Based on these experiments and previous data (Green et al., 1986), SPD has several characteristics that indicate the potential for biological control of coyotes in areas where population reduction is indicated. First, indigenous SPD is geographically and environmentally restricted to coastal California, Oregon and Washington in areas where the snail intermediate host, *O. silicula*, occurs. Therefore, the rickettsiae could probably be used in other areas without multiplication of organisms in the environment. Second, the host range for fatal effects of disease is limited to canids, and domestic dogs that inadvertently eat infected fish can be treated effectively with tetracycline resulting in a long term immunity. Third, SPD may be more humane than chemical and mechanical methods of coyote control that are currently used, and appears to be safer than chemical toxicants because of the narrow host range and the short term survival in the environment. Further research is needed to fully investigate the potential of SPD as an alternate method of coyote control in areas where control methods currently employed are considered unacceptable.

ACKNOWLEDGMENTS

We are grateful to Steve Evans, Denis Pochock, Don Beard and other Washington De-

partment of Fisheries personnel for their cooperation in this project. We also acknowledge and appreciate the excellent animal care provided by Kris Foreyt who bottle raised most of the coyote pups, and Laboratory Animal Research Care personnel at Washington State University. The laboratory assistance of Daniel Rice and Davis Bendezu is also greatly appreciated.

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Received for publication 21 July 1986.