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## EXPERIMENTAL INFECTION OF *CONTRACAECCUM MULTIPAPILLATUM* (NEMATODA: ANISAKINAE) FROM MEXICO IN THE DOMESTIC CAT

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**ABSTRACT:** Juveniles of *Contracaecum multipapillatum* infected the Mayan cichlid (*Cichlasoma urophthalmus*) and adults infected the olivaceous cormorant (*Phalacrocorax olivaceus*) and the great egret (*Casmerodius albus*) in the coastal lagoon at Celestun, State of Yucatan, Mexico. All are new host records, and, even though the geographic locality record of Mexico for the species has not been published, unidentified but presumably conspecific specimens have been reported from there. When juveniles of *C. multipapillatum* were fed to a kitten, but not rats, ducks, or chickens, they developed into adults. Measurements and morphological data are provided on the specimens from the kitten. Development of an avian ascaridoid in the intestine of a mammal increases the potential of this widespread species to infect other mammals, including humans.

*Contracaecum multipapillatum* infects several piscivorous birds in the stork, pelican, cormorant, anhinga, and heron families in the Americas, but, based on reports of juveniles from fishes, the species may extend much farther, even to the Middle and Far East (Deardorff and Overstreet, 1980a). Moreover, adults have been confirmed in pelicans from Zaire (as Belgian Congo), Africa, by Ezzat and Tadros (1958). The species, like most of the numerous members of the genus *Contracaecum*, is not known to naturally infect terrestrial mammals, although a few species in the genus occur in marine mammals. The present paper describes an experimental study demonstrating the first case of an avian species of *Contracaecum* that can mature in a mammal and cautions about a potential public health problem.

### MATERIALS AND METHODS

In July 1988, 48 specimens of the euryhaline Mayan cichlid (*Cichlasoma urophthalmus*), a species commonly eaten by people in Mexico, were captured in the coastal lagoon at Celestun (20°51'N, 90°23'W) in the State of Yucatan, Mexico. The fish were transported live to the Laboratory of Parasitology at the Center of Research and Advanced Studies, Merida Unit (CINVESTAV) for analysis. Fourth-stage juveniles of *C. multipapillatum* were collected for experimental infections from 30 captive hosts and fed to 9 3-wk-old chickens (*Gallus gallus*), 7 1-mo-old rats (*Rattus rattus*), 6 1-2-mo-old ducklings (*Anas platyrhynchos*) and a 2-mo-old kitten (*Felis catus*).

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Except for the kitten, these potential hosts were administered encapsulated worms orally. The number of juvenile worms fed to each animal varied from 11 to 36 for chicks, 7 to 28 for rats, and 35 to 50 for ducks. The chicks were killed and the alimentary tract was examined for worms at 48-120 hr (and in 1 case, 23 days), rats after 4-6 days, and ducks after 6-19 days. The kitten was from an ordinary domestic environment and maintained for 2 wk under laboratory conditions. It was fed exclusively uninfected raw muscle of *C. urophthalmus*, and its feces were inspected for parasitic eggs using a zinc sulfate flotation technique (MAFF/ADAS, 1988). There was no evidence of any parasites before the experimental infection. The cat was fed with the remaining 18, naturally infected specimens of *C. urophthalmus* every third day over a period of 33 days before being killed. The exact number of worms fed to the cat was not established. The chicks, rats, ducks, and kitten were maintained in an animal facility under constant 28 C conditions with a 12:12 hr light : dark photoperiod. All experimental hosts were killed with ether and death was demonstrated by corneal and pedal reflex tests (Waynforth, 1980). Terminology for infections follows that of Margolis et al. (1982).

### RESULTS

Of the 30 specimens of *C. urophthalmus* examined for *C. multipapillatum*, 43% were infected with an intensity of 1-18 juveniles, a mean intensity of 3.5 worms per infected host, and an abundance of 1.5 worms per fish examined. We also identified adult specimens of *C. multipapillatum* concurrent with *Contracaecum rudolphi* in the olivaceous cormorant (*Phalacrocorax olivaceus*) and the great egret (*Casmerodius albus*) in Celestun, Yucatan.

The encapsulated juveniles fed to chicks, rats, and ducks apparently did not survive to develop into adults because none was observed in the necropsied animals. Ten nematodes were recovered from the most anterior region of the intes-

tine of the cat. Included were 4 mature males, 1 gravid female, 3 nongravid females, and 2 juvenile females, confirming the identity of the species. At least 3 of those specimens were firmly attached to the intestine, causing hemorrhaging associated with small ulcers.

The males from the cat ranged from 19.8 to 29.8 mm in length with a width of 0.40–0.52 mm at the level of the ventriculus and lips approximately as long as wide. The subventral lips were about 88  $\mu\text{m}$  long by 88  $\mu\text{m}$  wide in the smallest male and 105 by 100  $\mu\text{m}$  in the largest, with the dorsal lip 85 by 108  $\mu\text{m}$  in the smallest and 108 by 100  $\mu\text{m}$  (largest dorsal lip) in the next to largest specimen. The interlabia ranged from 64 to 85  $\mu\text{m}$  long. The length of the esophagus ranged from 2.96 to 3.48 mm long by 0.09–0.26 wide, or 14.9–11.7% of the total body length (TBL). The length of the intestinal cecum, 2.12–2.90 mm long, ranged from 3.5 to 4.6 times longer than the ventricular appendix, which was 0.54–0.83 mm long. The nerve ring was located 1.6–2.4% TBL from the anterior end, and the tail was 0.3–0.7% of TBL. The nearly equal spicules, 718–1,275  $\mu\text{m}$  long, ranged from 1.8 to 4.3% of TBL. There were 100–130 pairs of preanal papillae plus 11 additional posterior pairs. Of these, there was a group of 2 near-medial and 2 lateral near the tip of the tail. The phasmid was located between and just lateral to the most lateral ones. After a distinct and consistently occurring space anterior to that group, the next 2 pairs comprised a “double.” The next 5 were neither in a straight line nor consistently located among the specimens. In fact, 1 specimen lacked the dextral set of postanal papillae. Typically, the posterior 2 pairs were perpendicular to the axis of the worm, and the last 3 (adanal papillae) cupped around the anal area. Nevertheless, some of these “adanal” papillae appeared nearly continuous with the preanal papillae in 3 specimens. Minute ventral crests on the annules extended from the anal level to the anteriormost preanal papillae.

Five females ranged from 20.1 to 38.6 mm in length with a width of 0.40–0.65 mm at the level of the ventriculus and lips approximately as long as wide in the largest specimen. The subventral lips were about 61  $\mu\text{m}$  long by 70  $\mu\text{m}$  wide in the smallest female and 116 by 113  $\mu\text{m}$  in the largest, with the dorsal lip 44 by 71  $\mu\text{m}$  in the smallest. The interlabia ranged from 47 to 85  $\mu\text{m}$  long. The length of the esophagus ranged from 2.83 to 4.43 mm long by 0.13–0.17 mm wide, or 14.1–10.6% of the total body length (TBL).

The length of the intestinal cecum, 2.13–3.70 mm long, ranged from 2.1 to 5.5 times longer than the ventricular appendix, which was 0.53–0.67 mm long. The nerve ring was located 1.0–2.1% TBL from the anterior end, and the tail was 0.6–0.9% of TBL. The vagina was located 28.0 (in the smallest specimen) to 38.3% of TBL from anterior end and was 47–513  $\mu\text{m}$  long by 17–129  $\mu\text{m}$  wide. Eggs ( $n = 10$ ) averaged 53  $\mu\text{m}$  long by 38  $\mu\text{m}$  wide.

A pair of specimens from the kitten has been deposited in the collection of the Helminthology Laboratory of the Biology Institute of The National University of Mexico (Mexico D.F.) as UNAM Reference Number 195-4. All adult specimens were conspecific with *C. multipapillatum* as reported by Lucker (1941) and others, e.g., Barus (1966), in natural bird hosts and by Deardorff and Overstreet (1980a) in experimental hosts.

#### DISCUSSION

The specific observations of *C. multipapillatum* in *C. urophthalmus* and the 2 bird hosts (*P. olivaceus* and *C. albus*) have not been reported previously and neither has the geographic record of the nematode from Mexico. However, in an unpublished bachelor's thesis, Amaya-Huerta (1990) indicated that the species infected *Nycticorax nycticorax* and *P. olivaceus* in Teapa, Tabasco, Mexico. Moreover, Pearse (1936) reported immature “encysted” specimens of *Contracaecum* sp. in *Cichlasoma mayorum* in Xtoloc Cenote, Yucatan. Robert Rush Miller, the authority on Mexican cichlids (R. R. Miller, 1993, pers. comm.), considers *C. mayorum* (established as a subspecies) a junior synonym of *C. urophthalmus*. Pearse (1936) did not list his reported infection in his Table I, and he presented descriptive data on adult females rather than immature specimens. Nevertheless, he probably collected juvenile, encapsulated, unidentified specimens of *C. multipapillatum*. Furthermore, based on host, site in host, and illustrations of specimens, the nematode reported as *Contracaecum* sp. from *Mugil cephalus* in Manzanillo, State of Colima, Mexico (Pacific Ocean coast), by Salgado-Maldonado and Barquín-Alvarez (1978) could well be conspecific with our Mexican material and that of Deardorff and Overstreet (1980a) from the same host in Mississippi, Louisiana, and adjacent areas. As indicated in the review by Deardorff and Overstreet (1980a), what is probably the same species

of *Contracaecum* has been reported from various specific fish species, some of which include common seafood products. Other pelican and egret hosts related to those hosts noted in Mexico also have been reported, e.g., Deardorff and Overstreet (1980a).

We do not consider the presence of the species in Yucatan as unusual, especially since some avian hosts such as the cormorant migrate from Florida, Louisiana, and Mississippi into that area. Infections have been adequately reported from wide-ranging bird hosts nearby in Cuba (Barus, 1966).

Because this avian nematode can mature in the intestine of the domestic cat, it might possibly mature in or penetrate the alimentary tract of humans. Deardorff and Overstreet (1980a), also experimenting with juveniles of *C. multipapillatum*, tried to infect rats, chicks, and ducklings by gavage, and rats, white mice, hamsters, and chickens by surgical insertion. Of those animals administered worms orally, none contained specimens after 72 hr, suggesting their lack of susceptibility to infection. However, of those animals receiving worms by surgery, only rats with worms inserted in the abdominal cavity maintained viable specimens. Those worms developed to fifth stage by day 10, and encapsulated specimens were still alive at 9 mo. In contrast, however, the juveniles from *M. cephalus* and *Sciaenops ocellatus* fed unsuccessfully to mammals by Deardorff and Overstreet (1980a) were initially third stage and those in the present study from a cichlid were fourth stage. That additional development might aid in inducing infectiveness of the species to mammals. A stray dog in Nemuro, Japan, had a granulomatous lesion in the stomach containing 2 specimens identified as *Contracaecum* sp. and 3 identified as *Terranova* sp. (Kitayama et al., 1967). The specimens reported as *Contracaecum* sp. had developed lips, indicating that they were fourth-stage juveniles; however, illustrations showed an intestinal cecum shorter than or equal in length to the ventricular appendix, indicating the species could belong in the genus *Hysterothylacium* (see Deardorff and Overstreet, 1980b). Also, Fagerholm and Gibson (1987) suggested the possibility that *Contracaecum ogmorhini* described from otariid seals actually represents accidental infections of an avian form.

*Contracaecum osculatum* from marine mammals, administered orally as a third-stage juvenile from fish, was recovered as fourth-stage

specimens at 2–5 days postinfection from the stomach of both laboratory rats and hamsters. Adults developed from third-stage juveniles at least by day 42 when specimens were surgically introduced into the body cavity of laboratory rats (Fagerholm, 1988); he suggested that because juveniles of *Contracaecum* spp. typically occur in visceral organs rather than flesh, they are not eaten by humans as frequently as flesh-inhabiting ascaridoids like *Anisakis* spp., and consequently they have received little research attention.

The domestic cat is not a common test animal for ascaridoids. Of 6 domesticated 4-mo-old cats fed juvenile specimens of *Anisakis simplex* (as *Anisakis* sp.) by Bille and Andreassen (1970), none showed signs of infection, but 1 had 2 juveniles that penetrated the wall of the ileum. Apparently, Gibson (1970) placed by laparotomy third-stage juveniles of *A. simplex* into the stomach of laboratory rats where some molted into fourth-stage specimens and remained for 3–4 days. Some worms penetrated through the gastric wall where in the body cavity they appeared to develop quicker and to a greater degree than corresponding worms in the alimentary tract, but they did not reach adulthood.

Ascaridoids do not need the ability to mature in warm-blooded hosts to be able to infect mammals even though most zoonotic ascaridoids in humans and experimental mammalian hosts develop to maturity in mammals. Overstreet and Meyer (1981) reported *Hysterothylacium* type MB that matures in teleosts to embed in or traverse the stomach wall of albino Swiss mice, usually dying within a week, as well as to traverse the stomach wall of the rhesus monkey. Deardorff et al. (1983) reported a juvenile ascaridoid (*Terranova* type HA) that probably develops to maturity in an elasmobranch to invade without complete penetration into the stomach and intestine of the laboratory rat.

Even though infective juveniles of *C. multipapillatum* typically encapsulate in the liver, kidneys, and mesentery of their specific fish hosts, they could be located elsewhere or they could be left in an eviscerated fish to be eaten subsequently by humans. If the host is eaten raw, inadequately cooked, or smoked with an insufficient amount of heat, incorporated worms could produce an infection, e.g., Deardorff (1986) and Deardorff and Overstreet (1991). Also, infectivity in mammalian hosts could depend on the ambient temperature of the water where the fish are caught. Ko (1976) found that he could infect

kittens and monkeys with a juvenile gnathostome nematode (*Echinocephalus sinensis*) from an oyster (*Crassostrea gigas*) near Hong Kong, but those infections occurred with nematode specimens collected from late July to October only and not with specimens collected from September into July. That observation stimulated him (Ko, 1977) to acclimate oysters with juvenile worms at different temperatures before feeding them to kittens. Substantial infections at 18 hr occurred in kittens fed worms acclimated at 28 and 33 C, temperatures common in Yucatan, northern Gulf of Mexico, and other localities where *C. multipapillatum* is abundant. When specimens of the juvenile gnathostome were acclimated at 5, 15, and 20–24 C, few, if any, infected kittens.

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