Praziquantel Resistance in the Zoonotic Cestode *Dipylidium caninum*

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Abstract. *Dipylidium caninum* is a cosmopolitan cestode infecting dogs, cats, and humans. Praziquantel is a highly effective cestocidal drug and resistance in adult cestodes has not been reported. From 2016 to 2018, a population of dogs with cestode infections that could not be eliminated despite multiple treatments with praziquantel or epsiprantel was identified. Cases of *D. caninum* were clinically resistant to praziquantel and could not be resolved despite increasing the dose, frequency, and duration of treatment. Resistant isolates were identified and characterized by sequencing the 28S, 12S, and voltage-gated calcium channel beta subunit genes. Cases were only resolved following treatment with nitroscanate or a compounded pyrantel/praziquantel/oxantel product. Clinicians should be aware of this alarming development as treatment options for cestodes are limited in both human and veterinary medicine.

INTRODUCTION

Dipylidium caninum is a cestode parasite that commonly infects dogs and cats.[1] Definitive hosts become infected following ingestion of a metacestode in an arthropod intermediate host. Typical intermediate hosts are the fleas *Ctenocephalides felis* and *Xenopsylla cheopis*,[2] or potentially the lice *Trichodectes canis*[3] and *Felicola subrostratus*.[4] Humans, especially infants, can be definitive hosts for *D. caninum* following accidental ingestion of arthropods that contain the cysticercoid metacestode.

In veterinary medicine, control of *D. caninum* involves treating dogs and cats with praziquantel[5] or epsiprantel[6] alone, or in combination with other anthelmintics for broad-spectrum coverage.[7–10] Praziquantel is also used to treat human *D. caninum* infections after parasitological identification of the cestode.[11] In addition, control of ectoparasites (fleas and lice) aids in breaking the life cycle of the parasite and in preventing transmission of the cestode to the vertebrate host.[12–14] In veterinary medicine, control of *D. caninum* is more often focused on eliminating ectoparasite intermediate hosts.

Praziquantel is an important drug for the control of platyhelminth parasites. There are several reports of reduced praziquantel susceptibility and failed clearance of trematode infections caused by *Schistosoma mansoni* and *Schistosoma haematobium* in endemic areas.[15] To date, there have been no reports of resistance to praziquantel in adult cestodes and clinical efficacy has been 100%.[16] This report describes clinical resistance to praziquantel in the zoonotic cestode *D. caninum*.

MATERIALS AND METHODS

Case descriptions. In the Summer of 2016, a veterinarian in Iowa recovered several cestodes while performing physical examination on a litter of puppies before issuing a certificate of veterinary inspection that would allow for interstate movement of the dogs. These specimens were submitted to the Department of Veterinary Pathology, Iowa State University for identification. At that time, the proglottids were identified as *D. caninum*, based on gross morphological features and characteristic egg packets expressed from the proglottids in squash preparations. The laboratory conferred with the referring veterinarian and developed a plan for integrated parasite control involving sanitation, use of praziquantel, and application of multimodal flea control.

Subsequently, between 2016 and 2017, the laboratory received inquiries from veterinarians in four different states (Colorado, Iowa, Michigan, and Minnesota) regarding cestodes that were refractory to praziquantel treatment at label doses ($\geq$ 5 mg/kg). Four of the cases originated from the population we examined in our initial study and one was a dog that originated in Florida and now resides in Iowa. The first four cases were young dogs, less than 2 years of age, whereas the fifth dog was middle-aged, approximately 5 years of age. All referring veterinarians were administering adequate flea control by administration of topical parasiticides and/or oral isoxazolines, and no fleas or flea dirt (digested blood indicating infestation) were found on any of the dogs. All treatments were administered by a veterinarian whenever possible.

Treatments. *Case 1.* Michigan (Pomeranian). The patient was administered oral epsiprantel (5.5 mg/kg), injectable praziquantel (5 mg/kg), oral praziquantel (10 mg/kg), and a combination praziquantel/pyrantel/febantel product daily for 8 weeks. The patient continued to shed proglottids posttreatment and was lost to follow-up after changing veterinarians.

Case 2. Colorado (Pomeranian). Before identification of the cestode, the patient received oral fenbendazole (50 mg/kg) once daily for 5 days and oral praziquantel (10 mg/kg) once daily for 2 days. At this time, oral heartworm prevention once monthly (2.3 mg milbemycin oxime with 22.8 mg praziquantel) was started. Next, a course of oral praziquantel (10 mg/kg) once weekly for 6 weeks was prescribed. The patient then received oral praziquantel (10.8 mg/kg), pyrantel (10.8 mg/kg), and febantel (54 mg/kg) once daily for 3 days. After no improvement, 2 weeks later, the dosages were increased to oral praziquantel (20 mg/kg), pyrantel (20 mg/kg), and febantel (108 mg/kg) once weekly for 2 weeks. With no improvement, 2 weeks later, an oral dose of nitroscanate (47 mg/kg) was administered. Oral nitroscanate was administered again (84 mg/kg) 2 weeks after the initial dose. No clinical side effects associated with the increased dose were noted and proglottids were no longer observed.

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Case 3. Iowa (Pomeranian). The patient was administered oral or injectable praziquantel (5 mg/Kg) at three veterinary visits, followed by double dose or oral praziquantel (10 mg/kg). The patient was then administered weekly praziquantel (5 mg/kg) for 6 weeks and daily praziquantel for 3 days. Following treatment with a compounded pyrantel/praziquantel/oxantel product daily for 3 days, proglottids were no longer observed.

Case 4. Minnesota (Pomeranian). The patient was administered oral and injectable praziquantel (5 mg/kg) at different veterinary visits, and then weekly for 6 weeks, followed by praziquantel (5 mg/kg) daily for 2 weeks. The dog continued to shed proglottids intermittently for approximately 4–5 months until the shedding spontaneously ceased.

Case 5. Iowa (Jack Russell Terrier). The patient was administered oral epsiprantel (5.5 mg/kg), oral epsiprantel (5.5 mg/kg) weekly for 7 weeks, injectable praziquantel (5 mg/kg) and oral epsiprantel (5.5 mg/kg) weekly for 4 weeks, epsiprantel orally followed by epsiprantel every 3 days for 12 days, and epsiprantel (11 mg/kg) and injectable praziquantel (10 mg/kg) every 3 days for 12 days, and continued to shed proglottids. Following treatment with a compounded pyrantel/praziquantel/oxantel product daily for 5 days, proglottids were no longer observed.

Molecular characterization. Proglottids were received from three of the five cases (Cases 1, 2, and 5) for identification and analysis. Proglottids were preserved in ethanol and examined for gross morphological features. Molecular characterization was also performed. Cestode DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Valencia, CA). A fragment of the nuclear rRNA encoding 28S gene that encodes the large ribosomal subunit was amplified and sequenced using the primers 5′-GCATGCAAGTCAAAGGGT CCTACG-3′ and 5′-CACATTCAACGCCCGACTCCTGTAG-3′.[17] A fragment of the mitochondrial rRNA encoding 12S gene was amplified and sequenced using the primers 5′-TTAA GATATATGTGGTACAGGATTAGATACCC-3′ and 5′-AACC GAGGGTGACGGGCGGTGTGTACC-3′.[18] Nucleotide basic local alignment search tool (BLAST) was carried out against the nonredundant nucleotide collection of GenBank, and maximum likelihood (ML) trees for both genes were constructed in Mega7[19] with the best-fit models determined using Bayesian information criterion calculations in jmodeltest 2.1.10.[20]

In addition, a fragment of the voltage-gated calcium channel beta subunit was amplified using the degenerate primers 5′-AAYAAYGAYGGTGGAT-3′ and 5′-GCYTTYTGCATCATRTC-3′,[21] cloned, and sequenced. BlastX search tool was used to compare the translated protein against the nonredundant protein sequence database on GenBank, and a neighbor-joining tree with the maximum composite likelihood model was constructed using Mega7.[19]

RESULTS

Gravid proglottids were approximately 1 cm in length, white, and tapered slightly at each end to give a "cucumber seed" appearance. Genital pores were located laterally. When crushed between glass slides, egg packets could be expressed from the proglottids, and measured 120–200 µm. Many hexacanth eggs in clusters were embedded in the egg capsule (Figure 1). Thus, all submissions were typical proglottids of the *D. caninum*.

BLASTn analysis of a 337 fragment of the mitochondrial 12S sequences revealed 100% identity between the three
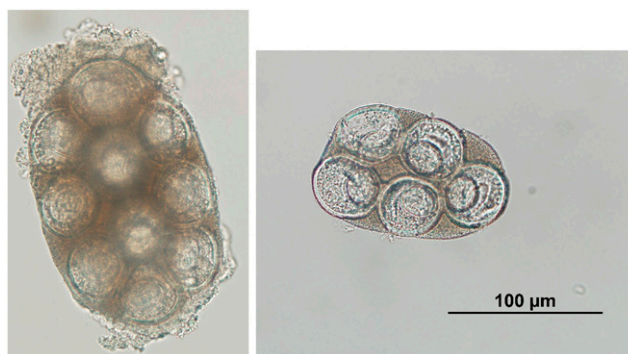

FIGURE 1. Egg capsules. Hexacanth embryos embedded in egg capsules seen in crush preparations of proglottids. This figure appears in color at www.ajtmh.org.

sequences, 100% identity with some isolates of *D. caninum* (AB732959 and AB031362), and 88–89% identity with other isolates (KY751955, KF202097, and L49460). BLASTn analysis of a 632-bp fragment of the nuclear 28S sequences revealed 100% identity between them, 99.4% identity with *D. caninum* (AF023120), and 94–95% identity with other isolates (MG575740, MG575741, MG575742, MG575743, and KY751956). 12S and 28S sequences from this study were deposited in GenBank (accession numbers MH182479–MH182481 and MH182476–MH182478, respectively).

The best-fit models for the 12S and 28S ML trees were determined to be Hasegawa–Kishino–Yano with gamma distribution (HSY + G) and equal frequency Tamura-Nei models, respectively. *Echinococcus multilocularis* 12S (AB018440) and *Raillietina australis* 28S (AF286914) were used as outgroups. In both ML trees, two distinct clades were distinguishable, and all *Dipylidium* isolates from the present study clustered within a single clade in both trees (Figures 2 and 3).

A 713-bp fragment of the voltage-gated calcium channel beta subunit was cloned from genomic DNA, spanning an exon–intron boundary of the gene. Voltage-gated calcium channel sequences from this study were deposited in GenBank (accession numbers MH189719–MH189720). BLASTx analysis revealed that the sequence encodes a part of the Beta interacting domain (BID) of the voltage-dependent L-type calcium channel beta-1 subunit, and included the Src homology 3 domain, typical of the BID. The translated amino acid sequence had the following identities with various voltage-gated calcium channel beta subunits: 81% with *Taenia solium* (AAV31726); 89% identity each with *Echinococcus granulosus* (EUB60654 and CDS23505), *E. multilocularis* (CDS39653), and *Hymenolepis microstoma* (CDS33662); and 82% identity with *Schistosoma japonicum* (AAX28365) and *S. mansoni* (AAQ19186). In the neighbor-joining tree of voltage-gated calcium channel beta subunit sequences, the cloned fragments from *Dipylidium* cluster with sequences from *Hymenolepis nana* (Figure 4).

DISCUSSION

A number of cestodes parasitize human definitive hosts, including selected species of *Diphyllobothrium*, *Spirometra*, *Taenia*, *Hymenolepis*, and *Dipylidium*.[22] *Dipylidium caninum* is most commonly found in dogs and cats, but can infect humans if they ingest arthropods containing the cysticercoid metacestode. Generally, *Dipylidium* infections induce mild to
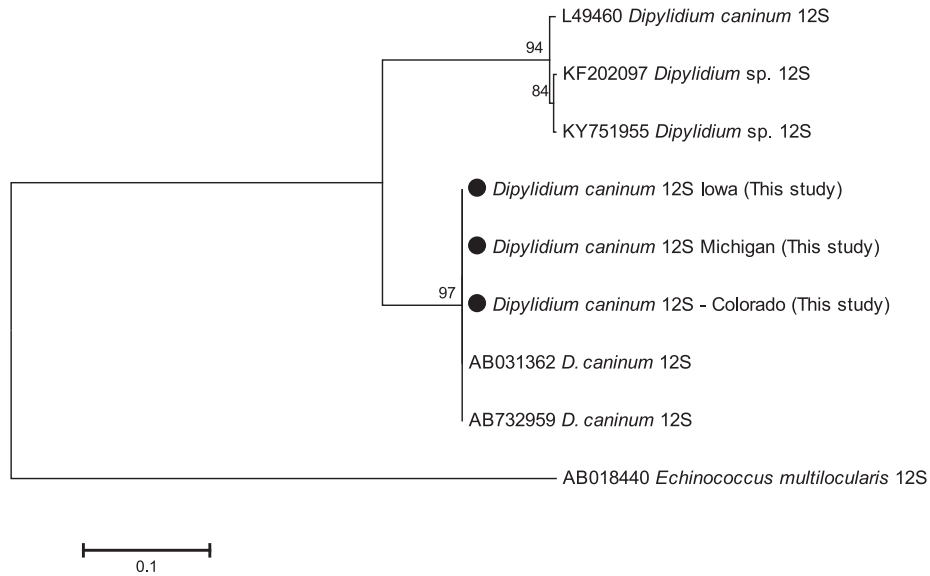
FIGURE 2. Maximum likelihood tree—mitochondrial 12S gene. The evolutionary history was inferred by using the maximum likelihood method based on the Hasegawa–Kishino–Yano model with a discrete Gamma distribution to model evolutionary rate differences among sites. The analysis involved nine nucleotide sequences and a total of 295 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

no clinical signs in dogs and cats. When signs occur, they may include abdominal pain, vomiting/diarrhea, and may be accompanied by a mild anal pruritus associated with proglottids in the perianal area. In humans, *D. caninum* infections occur occasionally, mainly in children who acquire the infection by ingesting intermediate hosts such as fleas. In this report, we document *D. caninum* that is clinically resistant to praziquantel, the mainstay of anti-cestode therapy.

A combination of classical parasitology and molecular techniques was used to conclude that the infections were caused by *D. caninum.* One drawback of this study was the inability to establish the resistant clinical isolates in experimentally infected laboratory animals to further study them. Reliable experimental *Dipylidium* infection in dogs using a flea infestation model has only recently been established[13,14] and involves rearing of flea larvae in classical flea-breeding units

with *D. caninum* egg packets for up to 2 weeks to allow for the development of the metacestode in the fleas before ingestion by dogs or cats.

In our investigation, none of the five cases could be resolved with label doses of praziquantel alone. Likewise, these infections could not be treated with epsiprantel, a closely related isoquinolone. Attempts using higher doses and durations of treatment also failed. Praziquantel has relatively low host toxicity and no side effects were associated with extended treatments. Often, reports of drug resistance in veterinary parasites can be associated with poor client compliance whereby medications are not given appropriately. In the present cases, treatments were administered by a veterinarian whenever possible. One of the infections was successfully treated with nitroscanate, two were successfully treated with a compounded pyrantel/praziquantel/oxantel product, one
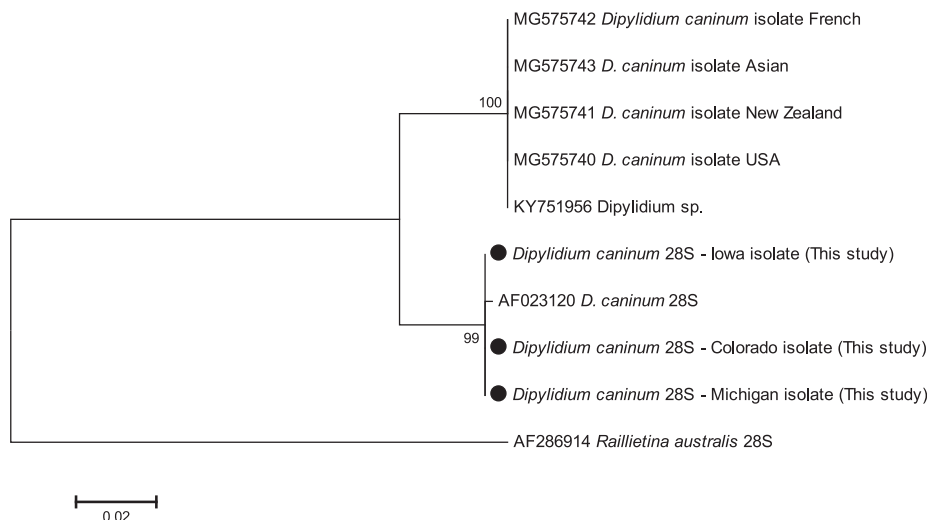

FIGURE 3. Maximum likelihood tree—nuclear 28S gene. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura–Nei model. The analysis involved 10 nucleotide sequences. There were a total of 580 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.
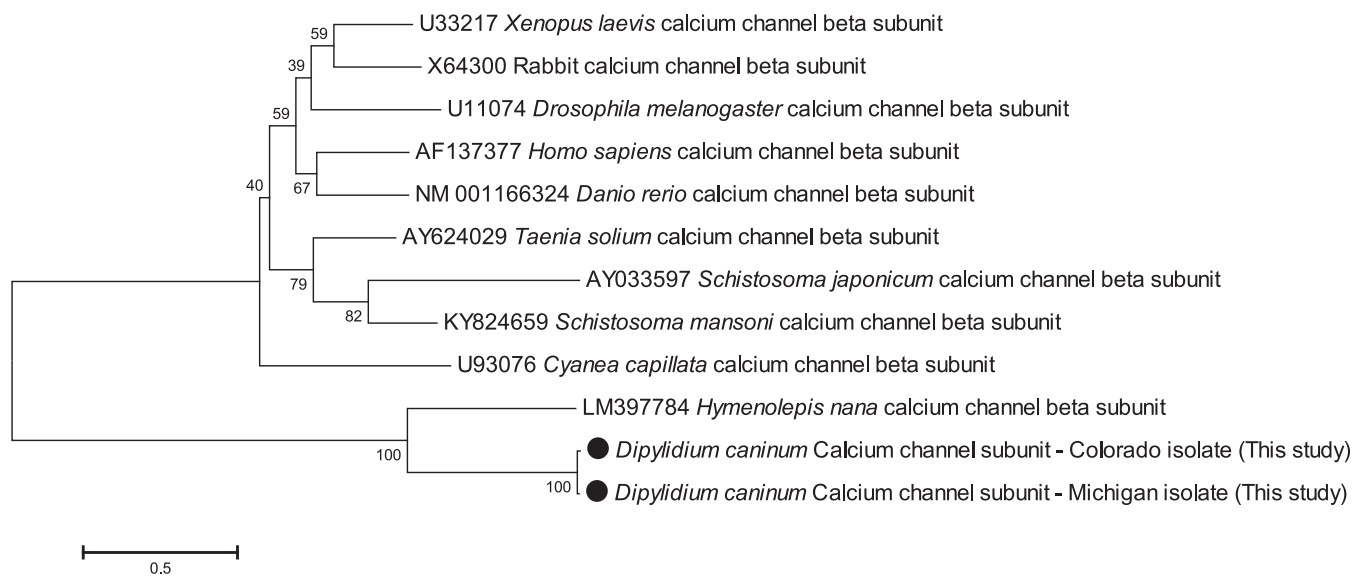
FIGURE 4. Neighbor-joining tree—calcium channel beta subunit. The evolutionary history was inferred using the neighbor-joining method, and evolutionary distances were computed using the maximum composite likelihood method. The analysis involved 12 nucleotide sequences. There were a total of 780 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

spontaneously ceased shedding proglottids after several months of treatment, and one was lost to follow-up. It is unclear why the compounded product was effective despite multiple failures of praziquantel and epsiprantel. It is possible that pyrantel acted synergistically, as there is evidence that it may act against some cestodes of veterinary interest.[23] Anecdotally, other dogs originating from the infected litter spontaneously ceased shedding proglottids following months of unsuccessful chemotherapy. It is possible that these cestodes were expelled following their natural lifetime, the duration of which is not well characterized.

The exact mechanism of praziquantel action is yet to be fully elucidated, and new targets are being studied to fully account for drug activity.[24] One well-studied and widely accepted target of praziquantel is the cestode voltage-gated calcium channels.[21] In our study, we cloned a partial sequence of the beta subunit of the voltage-gated calcium channel from the resistant cestodes, but analysis was constrained by the complete lack of availability of genome sequences of *D. caninum*. Future studies will assess the possibility that polymorphisms in the voltage-gated calcium channel account for clinical praziquantel resistance.

Praziquantel is the drug of choice for cestode infections,[25] and is the sole drug used on a large scale for human schistosomiasis.[26] In veterinary species, oral, topical, and injectable praziquantel formulations are available for use against cestodes and trematodes.[27] Despite the widespread use of praziquantel in human and veterinary medicine, examples of drug resistance in platyhelminthes are rare as compared with reports of drug-resistant nematodes.[28] These reports are more common in hyperendemic areas and are potentially associated with frequent exposure to the drug.[29,30] Although mass drug administration is common for control of veterinary parasites, and nematode drug resistance has become a serious problem, cestode drug resistance is not well documented.

This study is the first to report praziquantel resistance in *D. caninum*, a cestode with zoonotic importance. Future studies with clinically resistant isolates to elucidate the exact

mechanism of resistance are necessary, along with epidemiological studies to determine to determine the extent of the resistance problem. Practitioners should be aware of the presence of praziquantel-resistant *D. caninum* in the United States.

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