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Original Article

Redescription and first molecular characterization of the little known feline neurotropic nematode *Gurltia paralysans* (Nematoda: Metastrongyloidea)

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ABSTRACT

Gurltia paralysans is a poorly documented metastrongyloid nematode of cats, which mainly parasitizes the veins in the spinal cord subarachnoid space and parenchyma. Parasitic paraparesis caused by *G. paralysans* is a lesser-known spinal cord disease affecting domestic and wild felids of South America. Regions where feline gurltiosis is endemic include the southern parts of Chile and Argentina. *Intra vitam* diagnosis of feline gurltiosis remains challenging and is based primarily on neurological signs and the exclusion of other ethiologies for feline myelopathies. In view of the lack of information in the literature for this neglected feline neurological parasitosis, we have undertaken a detailed redescription and molecular characterization to expand on the previously available details in the original descriptions by Wolffhügel in 1993. The specimens used in this study were collected from spinal cord lesions of gurltiosis-affected domestic cats. Female and male specimens were morphologically and morphometrically examined using light and scanning electron microscopy. Molecular characterization was performed by sequencing a partial region of the nuclear ribosomal DNA and cytochrome oxidase gene of this parasite, and phylogenetic trees were constructed from the 28S D2-D3 and ITS2 regions using the Maximum Likelihood method. Sequence matching and phylogenetic analysis with these new sequences were consistent with the morphological classification of *G. paralysans* being within the Metastrongyloidea superfamily, but no consistent relation to a specific metastrongyloid family. The newly developed *G. paralysans*-specific PCR described here not only provides a useful diagnostic tool for feline gurltiosis in domestic cats living in endemic areas, but could also be used in large-scale epidemiological surveys on the intermediate mollusk host and the final host. By combining the morphology, molecular, and phylogenetic data we have reliably identified *G. paralysans* and confirmed its taxonomic status within the Metastrongyloidea.

1. Introduction

*Gurltia paralysans*Wolffhügel, 1933 is a poorly documented and emerging neurotropic metastrongyloid nematode of domestic cats in which the adult stages reside in the veins of the spinal cord subarachnoid space and parenchyma (Wolffhügel, 1933). The nematode *G. paralysans* was described by Wolffhügel (1933), who isolated some adult nematodes from the vein system of the leptomeninges of a domestic cat suffering from chronic pelvic paraparesis in the city of Valdivia, Chile. A year later, Wolffhügel (1934) published an account of this parasite, giving an extended description of its geographic distribution, morphology, pathological findings and clinical signs, and speculated on its transmission and definitive host spectrum. Wolffhügel (1934) also hypothesized that the main definitive hosts for the *G. paralysans* parasite in the south of Chile and the border region of Argentina was the South American wild felid kodkod (*Leopardus guigna*), which is known locally as 'guiña' but is referred by other names (e.g., spotted tiger cats) in publications by later authors (Skrjabin, 1961), and domestic cats (*Felis domesticus*) introduced into South American by European settlers (in modern times). Bowman (2009) suggested that the migration through the nervous tissue and paraparesis in domestic cats indicates that the natural final host for this parasite is the guiña (*L. guigna*), or another wild felid, the Geoffroy's cat (*Leopardus geoffroyi*).

The regions where feline gurltiosis is endemic are primarily the southern parts of Chile and its border with Argentina (Gómez et al., 2010; Moroni et al., 2012). More recent reports on clinical feline

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gurltiosis in Chile clearly show that adult *G. paralysans* nematodes are not restricted to within the vein system of the leptomeninges of the thoracic and lumbar spinal cord segments, because they have also been found in deep veins of the spinal cord in domestic cats (Gómez et al., 2010; Moroni et al., 2012; Moroni et al., 2016), where they occasionally cause severe myelopathies with a generally fatal outcome (Mieres et al., 2012). The clinical signs of feline gurltiosis can include chronic symmetrical or asymmetrical pelvic limb ataxia, ambulatory paraparesis, uni- or bi-lateral hyperactive patellar reflexes, proprioceptive deficit in the pelvic limbs, pelvic limb muscular atrophy (Gómez et al., 2010; Moroni et al., 2012; Togni et al., 2012; Mieres et al., 2013), diarrhea, weight loss, coprostasis (Wolffhügel, 1934), urinary and fecal incontinence, and even death (Wolffhügel, 1934; Bowman, 2009; Gómez et al., 2010). The mean age of the previously reported feline gurltiosis cases was 2.5 years (Gómez et al., 2010; Wolffhügel, 1933, 1934; Mieres et al., 2013) and, so far, 22 cases in domestic cats have been reported in the scientific literature (Gómez et al., 2010; Alzate et al., 2011; Guerrero et al., 2011; Rivero et al., 2011; Togni et al., 2012; Moroni et al., 2016). Live adult *G. paralysans* are often observed during necropsies (Supplementary video 1), and diffuse submeningeal venous congestion is a common pathologic finding (Gómez et al., 2010).

The main geographic distribution of feline gurltiosis is limited to the southern parts of Chile including mainly the woodlands of La Araucania, Los Lagos and the Los Rios regions of Chile (Wolffhügel, 1933; Gómez et al., 2010; Moroni et al., 2012), and the border with Argentina (Wolffhügel, 1934; Gómez et al., 2010; Moroni et al., 2012). *G. paralysans* has also been found sporadically in domestic cats from Uruguay, Colombia, and Brazil (Alzate et al., 2011; Rivero et al., 2011; Togni et al., 2012). Additionally, recent reports have described the isolation of *G. paralysans* from adult wild felids (*Leopardus triginus* and *Leopardus wieddi*) in Brazil (Dazzi et al., 2015; Oliveira, 2015). It has been speculated that the disease might also be present in the central parts of Chile, where *L. guigna* is also endemic. The epidemiology of the disease is still largely unknown and detailed studies on its prevalence in geographically endemic areas are still unrecorded. Thus, feline gurltiosis might still be underdiagnosed and its real prevalence underestimated.

The main objectives of this study were to update the morphological description of the adult nematodes using light and scanning electron microscopy (SEM) and to perform a sequencing study for molecular characterization and phylogenetic relationships. We also established a PCR-based molecular diagnostic method that can be used in future research related to the epidemiology and life-cycle related aspects of feline gurltiosis.

2. Materials and methods

2.1. Specimens and measurements

Seven males (one slightly damaged), fourteen male posterior sections and two female *G. paralysans,* preserved in 70% alcohol, previously isolated from domestic cats originally from Paillaco County in Southern Chile were available for this study. The males and two females previously assigned to the Parasite Collection of The Royal Veterinary College (RVC), London, United Kingdom (number 8064) were transferred to the Natural History Museum collection (London, United Kingdom), where all additional material has been deposited. After cleaning with lactoglycerol (equal parts glycerol, lactic acid and distilled water), the specimens were temporarily mounted using the same solution. Measurements were taken from two undamaged males and the two females after using the cleaning agent, except for the body lengths, which were made prior to removal of the 70% ethanol. The cephalic sensory papillae details were taken from the SEMs. All measurements were made from specimens in the cleaning agent, except the body lengths, which made in the presence of ethanol.

2.2. SEM

The two adult female and two adult male *G. paralysans* nematodes previously isolated from the vein system of the leptomeninges of necroptized *G. paralysans*-infected domestic cats by Gómez et al. (2010) at the Faculty of Veterinary Sciences, University Austral of Chile (Chile), were analyzed in more detail by SEM. Briefly, the specimens were placed on poly-$_{L}$-lysine pre-coated glass coverslips (10 mm diameter) and thereafter fixed (2.5% glutaraldehyde in 0.1 M cacodylate buffer, Merck, Rahway, NJ). The specimens were then post-fixed in 1% osmium tetraoxide in 0.1 M cacodylate buffer (Merck), washed three times in distilled water, dehydrated with ascending ethanol concentrations, critical point-dried with CO_2, and afterwards coated with gold particles. The specimens were examined using a Philips 30 M scanning electron microscope (Philips, Eindhoven, The Netherlands) at the Institute of Anatomy, Faculty of Veterinary Sciences, University Austral of Chile.

2.3. DNA extraction and development of a G. paralysans-specific semi-nested PCR

The adult male and female *G. paralysans* nematodes that were previously isolated during necropsies from domestic cats suffering gurltiosis with progressive paraparesis and paraplegia from the lumen of leptomeningeal vein vessels at the Institute of Animal Pathology, Faculty of Veterinary Sciences, University Austral of Chile were used. All the specimens were immediately washed after extraction in sterile phosphate-buffered saline, fixed in 70% ethanol, and then maintained at − 80 °C. The fixed specimens were transferred to the Institute of Parasitology, Justus Liebig University Giessen (Germany) for molecular analysis. After equilibration of the worms in sterile water, DNA was extracted according to the instructions of the DNeasy blood and tissue kit® (Qiagen, Hilden, Germany). Additionally, *Aelurostrongylus abstrusus* DNA was extracted from adult male and female nematodes for inclusion in the molecular analysis. Briefly, each single nematode specimen was added to 1.5 mL tubes and then lysed by adding 180 µL of ATL buffer and 20 µL of Proteinase K (20 mg/mL) followed by overnight incubation at 56 °C with vigorous shaking on a vortex apparatus until complete lysis was achieved. Then, 200 µL of ATL buffer and 200 µL of 96% ethanol were added to each tube and the solution was transferred to DNeasy Mini columns (Qiagen) and centrifuged at 800 × *g* for 1 min. After washing the column-bound DNA with buffers AW1 and AW2 the column was centrifuged at full speed for 3 min and the DNA was eluted with 50 µL of AE buffer (800 × *g*, 1 min). The extracted *G. paralysans* and *A. abstrusus* DNA was quantified by spectrophotometry.

A partial sequence of the *G. paralysans* ribosomal region including internal transcribed spacer 1 (ITS1), 5.8S rRNA gene, ITS2 and partial sequences of the18S and 28S rRNA genes was amplified using standard PCR conditions and universal primers 652 5′-GCAGCCGCGGTAATTCCAGCTC-3′ (Nadler et al., 2007), NC2 5′- TTAGTTTCTTTTCCTCCGCT-3′ (Gasser et al., 1993), NC5 5′- GTAGGTGAACCTGCGGAAGGATCATT-3′ (Gasser and Hoste, 1995), and D3B 5′-TCGGAAGGAACCAGCTACTA-3′ (Nunn, 1992). A partial *G. paralysans* mitochondrial cytochrome oxidase subunit 1 gene sequence (cox1) was amplified with a metastrongylid adapted LCO1490 (Folmer et al., 1994) primer MetCOIF1 5′-GARAGTTCTAATCATAAGGATATTGG-3′and the universal primer JB4.5 5′-TAAAGAAAGAACATAATGAAAATG-3′ (Bowles et al., 1992).

The new *G. paralysans* sequences were submitted to GenBank under accession numbers JX975484 and KM245572.

To differentiate between *G. paralysans*-specific DNA and DNA originating from *A. abstrusus*, two species-specific semi-nested PCRs based on the 5′-end of the 28S rRNA gene were designed with the program Beacon designer (Premier Biosoft, Palo Alto, USA). The first PCR amplified DNA from both species using universal oligonucleotides (forward: AaGp28Ss1 5′-CGAGTRATATGTATGCCATT-3′, reverse: