Characterization of a New Species of the Ciliate Tetrahymena (Ciliophora: Oligohymenophorea) Isolated from the Urine of a Dog: First Report of Tetrahymena from a Mammal

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Summary. An approximately 10 yr-old male Dalmation was admitted to the Exeter Animal Hospital presenting symptoms of continuous urination, polyuria/polydypsia, and regurgitation. Urinalysis showed glucosuria, pyuria, proteinuria, casts, and white blood cells. Microscopic examination of urine revealed considerable numbers of a ciliated protozoan. The ciliate was isolated and aseptically cultured in proteose peptone medium. Cytological staining of cells with the Chatton-Lwoff silver nitrate and silver proteinate procedures demonstrated that the ciliate was a species of the genus Tetrahymena, measuring about 50 x 25 µm, placing it within the “pyriformis” species complex. Polymerase chain reaction amplification of the small subunit rRNA (SSrRNA) gene followed by DNA sequence analysis confirmed this identification. Analysis of the complete SSrRNA gene demonstrated significant differences in primary sequence from all other members of the “pyriformis” species complex and justified the designation of a new species, Tetrahymena farleyi sp. n.

Key words: polymerase chain reaction, small subunit rRNA sequence, Tetrahymena farleyi sp. n., urinary infection.

INTRODUCTION

Ciliated protozoans are symbionts of a wide variety of animals, both invertebrate and vertebrate (Corliss 1979). Typically, they are commensal organisms, neither helping nor harming their host. There is some evidence that the ruminant ciliates may be mutualistic, enhancing the growth and weight gain of their ruminant hosts while themselves benefiting from the rumen environment (Hungate 1966). A minority of the species has been designated as parasitic or facultatively parasitic (Corliss 1979). Balantidiuim, a member of the Class Litostomatea (Subclass Trichostomatia), has long been considered the only parasitic ciliate of vertebrates (Corliss 1979), typically invading the intestinal tissues of human beings and their domestic and other animals (Zaman 1978). However, a recent report has recorded Balantidium in the urinary bladder of a human being (Maleky 1998). Facultative parasites are common in the Class Oligohymenophorea, especially the Subclasses Scuticociliatia and Hymenostomatia (Hoffman 1978). Several genera of scuticociliates have been reported to infect mostly marine organisms, including oysters (Elston et al. 1999), crabs (Morado and Small 1995, Messick and...
Small 1996), lobsters (Cawthorn et al. 1996), and fishes (Hoffman 1978, Cheung et al. 1980). The hymenostome ciliates Tetrahymena, Ophryoglena, and Ichthyophthirius are reported to parasitize a wide variety of freshwater organisms. Ichthyophthirius is an obligate parasite of fishes often resulting in the death of the host fish (Hoffman 1978, Corliss 1979) while Ophryoglena is mainly an opportunistic histophagous on moribund or dead invertebrates (Corliss 1979).

Species in the genus Tetrahymena and its phylogenetically close relative Lambornella are also typically characterized as facultative parasites. They have been reported to infect and cause the death of invertebrates, such as black flies (Corliss et al. 1979, Lynn et al. 1981, Batson 1983), mosquitoes (Washburn et al. 1988, Jerome et al. 1996), and vertebrates, especially fishes (Hoffman et al. 1975, Hoffman 1978, Ferguson et al. 1987). To our knowledge, there has never been a report of a Tetrahymena infection of a mammal.

The present study was undertaken when a Dalmation dog was admitted to the Exeter Animal Hospital with an infection of the urinary system. Microscopic observation of the urine revealed numerous ciliated protozoa swimming freely in the fluid. These ciliates were tentatively identified as members of the genus Tetrahymena on initial examination. To confirm this preliminary identification, the ciliates were cultured in sterile medium, stained for cytological examination, and the small subunit rRNA (SSrRNA) gene was amplified by the polymerase chain reaction (PCR). These further characterizations corroborated the ciliate as a Tetrahymena and moreover indicated that this was a new species, Tetrahymena farleyi sp. n., which is described in detail herein.

MATERIALS AND METHODS

The agent

This isolate of Tetrahymena was obtained from the urine of a Dalmation dog admitted for euthanasia on 25 September, 1996 to the care of Dr. Ellen Shapiro, Exeter Animal Hospital, 660 Main Street South, Exeter, ON N0M 1S1, Canada. No information is available about how the dog acquired the infection.

Culture techniques

The ciliates were isolated in 0.05% Cerophyl culture medium inoculated with Enterobacter aerogenes. Clonal isolates were established in proteose peptone-yeast extract (PPYE) medium (0.5% proteose peptone, 0.5% yeast extract, 0.125% dextrose anhydride) on 15 April 1998 and have been submitted to the American Type Culture Collection with Accession No. 50748. Isolates have been propagated by biweekly transfers since that time.

Cytological techniques

Ciliates were pelleted from the Cerophyl medium by centrifugation. They were then fixed in Champy’s Fluid followed by Da Fano’s Fluid, and stained by the Chatton-Lwoff silver nitrate technique (Foissner 1992) or fixed in Bouin’s Fluid and stained using a silver proteinate stain (Montagnes and Lynn 1993).

Polymerase chain reaction

Isolation of DNA and PCR amplification of the SSrRNA genes followed procedures described in Jerome and Lynn (1996).

DNA sequencing and phylogenetic analysis

PCR-amplified DNA was purified on 1.0% agarose gels using the GeneClean® Kit. The SSrRNA was sequenced directly in both directions with an ABI Prism 377 Automated DNA Sequencer (Applied Biosystems Inc.) using dye terminator and Taq FS with three forward and three reverse internal universal 18S primers (Elwood et al. 1985).

Sequence availability and systematic analysis

The nucleotide sequences in this paper are available from the GenBank/EMBL databases under the following accession numbers: Tetrahymena australis, M98015, X56167; Tetrahymena borealis, M26359, M98020; Tetrahymena canadensis, M26359, M98022, X56170; Tetrahymena capricornis, M98018, X56172; Tetrahymena hegewischi, M98019, X56166; Tetrahymena hyperangularis, M98014, X56173; Tetrahymena malaccensis, M26360; Tetrahymena nanneyi, M98016, X56169; Tetrahymena patula, M98017, X56174; Tetrahymena pigmentosa, M26358; Tetrahymena pyriformis, M98021, X56171; Tetrahymena tropicalis, M98023, X56168 (Sogin et al. 1986); Tetrahymena thermophila, M10932 (Spangler and Blackburn 1985); Tetrahymena corlissi, U17356 (Wright and Lynn 1995); Tetrahymena empidokryeia, U36222 (Jerome et al. 1996). All sequences were globally aligned using the Dedicated Comparative Sequence Editor (DCSE) program (De Rijk and De Wachter 1993) and further refined by considering secondary structural features of the 18S molecule. Nucleotide differences between species were then tabulated.

RESULTS

Evaluation of the dog

An approximately 10 yr-old, neutered male Dalmation, named “Farley”, was admitted presenting symptoms of continuous urination, polyuria/polydypsia, and regurgitation. The dog weighed about 27 kg and had been losing weight for about one month: the owner reported the normal weight to have been around 36 kg. Blood glucose was > 500 mg/dl, suggesting diabetes mellitus. The
bladder was thickened but there were no palpable stones. Urine was collected midstream. It was deep yellow in colour, aromatic, and turbid. Urinalysis showed a specific gravity of 1.023, glucosuria, pyuria, proteinuria, casts, white blood cells, and ciliates.

Immediately after the dog was euthanized, the urine sample was collected aseptically via aspiration through the bladder and abdominal wall. The urine sample was then used as the source to establish the clonal cultures of the ciliate.

**Cytology of ciliate**

The ciliate was ovoid to pyriform in cell shape. Both silver stains revealed an oral apparatus typical of the genus *Tetrahymena*: there were three oral membranelles on the left side of the oral cavity and an undulating membrane on the right side. The cell body was covered by ciliary rows evenly spaced over the cell surface (Fig. 1). After Chatton-Lwoff staining, the following measurements characterized the ciliate: body length - 48.0 (42-54.3) ± 3.5 µm (mean - range ± standard deviation) (n = 31); body width - 19.4 (14-24.5) ± 2.4 µm (n = 31); oral cavity length - 10.4 (8.8-12.3) ± 0.54 µm (n = 31); length of oral membranelle 1 - 5.7 (5.3-7) ± 0.65 µm (n = 26); length of oral membranelle 2 - 4.9 (3.5-5.3) ± 0.60 µm (n = 26); length of oral membranelle 3 - 2.5 (1.8-3.5) ± 0.53 µm (n = 26); number of somatic kineties or ciliary rows - 16 (15-17) ± 0.58 (n = 28); and two postoral kineties (n=31). After QPS staining, the following measurements characterized the ciliate: body length - 50 (43.8-56) ± 3.1 µm (n = 30); body width - 21.8 (17.5-26.3) ± 2.29 µm (n = 30); macronuclear length - 11.4 (8.8-14.9) ± 1.27 µm (n = 30); and macronuclear width - 10.5 (8.8-14) ± 1.01 µm (n = 30). The ciliate did not appear to have a micronucleus nor a caudal cilium.

**PCR and sequence analysis of ciliate**

PCR amplification yielded a PCR fragment of approximately 1,700 bp in length. Complete sequence of the SSrRNA from this ciliate was 1749 nucleotides and is deposited under Accession Number AF184665. It differed at least one of a total of 64 positions from the other species of the “*pyriformis*” species complex. It differed from all other species at two positions: it had a T rather than a C at nucleotide positions 700 and 1663 of *Tetrahymena thermophila* (Table 1). Phylogenetic analysis (data not shown) indicated that it was most closely related to *Tetrahymena tropicalis* with which it showed the fewest differences in sequence (Table 1).

**Description of new species**

On the basis of these sequence differences, we justify the establishment of a new species in the genus *Tetrahymena*.

*Tetrahymena farleyi* sp. n. (Fig. 1, Table 1)

**Etymology:** named after “Farley”, the Dalmation dog from which the isolate was derived.

**Type locality:** near Exeter, ON, Canada (48° 21’N, 81° 29’W)

**Description:** *Tetrahymena farleyi* sp. n. is ovoid to pyriform in cell shape, and morphologically indistinguishable from previously described species in the “*pyriformis*” species complex. *Tetrahymena farleyi* sp. n. cultured in PPYE ranged in body length from 42 - 56 µm and in body width from 14 - 26 µm, with 15 - 17 somatic kineties with two postoral kineties. This species is amicronucleate and lacks a caudal cilium.

Type specimens: a type culture of *T. farleyi* sp. n. (Accession No. 50748) has been submitted to the Ameri-
Table 1. Basepair differences between *T. farleyi* and some other *Tetrahymena* species

<table>
<thead>
<tr>
<th>Sequence Position 5' → 3'</th>
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<tbody>
<tr>
<td>Species</td>
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<tr>
<td><em>T. farleyi</em></td>
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<tr>
<td><em>T. australis</em></td>
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<td><em>T. borealis</em></td>
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<td><em>T. capricornis</em></td>
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<td><em>T. corlissi</em></td>
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<td><em>T. hegewischi</em></td>
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<td><em>T. malaccensis</em></td>
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<td><em>T. empidokyrea</em></td>
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<td><em>T. nanneyi</em></td>
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<td><em>T. patula</em></td>
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<tr>
<td><em>T. pyriformis</em></td>
</tr>
<tr>
<td><em>T. tropicalis</em></td>
</tr>
<tr>
<td><em>T. thermophila</em></td>
</tr>
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1 *T. borealis* and *T. canadensis* have the same sequence; 2 *T. nanneyi*, *T. hyperangularis*, and *T. pigmentosa* have the same sequence.
can Type Culture Collection (Manassas, VA, USA). A Chatton-Lwoff stained type slide (USNM) of *T. farleyi* sp. n. has been submitted to the Ciliate Type Slide Collection of the National Museum of Natural History (Smithsonian Institution, Washington, D.C., USA).

**DISCUSSION**

This is the first report of a *Tetrahymena* species from a mammal. It is not known whether the ciliate infection was a primary or secondary one, responsible for the bladder infection of this dog. *Tetrahymena* infections of invertebrates (Corliss *et al.* 1979; Batson 1983, 1985) and vertebrates (Thompson 1958, Hoffman *et al.* 1975, Ferguson *et al.* 1987) are known to be fatal. Therefore, it is not unreasonable to suppose that the extreme condition of this dog was caused by the ciliate infection in the bladder.

Not only is this the first report of a *Tetrahymena* infection in a mammal, the isolate also appears to be a new species of *Tetrahymena*, which we have named *Tetrahymena farleyi* sp. n. Since this species was maintained for several months on a bacterized culture medium, it must be classified as a facultative parasite. It is not known how this ciliate came to infect the dog nor whether this ciliate species has a preference for mammalian hosts.

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**REFERENCES**


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