

Short communication

First Report of a Case of Prostatitis Due to *Acanthamoeba* in a Dog

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Abstract. The first case of prostatitis in a ten year old mixed breed dog due to *Acanthamoeba* genotype T4 is reported. The dog was suffering from kidney dysfunction and was admitted for exploration of its organs by echography. All organs were in normal conditions with the exception of the prostate which showed signs of inflammation. An ultrasound-guided puncture was thus performed for further cytological and microbiological study. When the obtained fluid was observed under the microscope, *Acanthamoeba* trophozoites were detected in a high number. No other pathogens were isolated. Both culture and PCR were positive for *Acanthamoeba* genus and the isolate was later identified as genotype T4. Unfortunately at this stage, the dog's owner decided to reject any kind of treatment or therapy. To the best of our knowledge, this is the first report of prostatitis in a dog due to *Acanthamoeba* genus.

Key words: Dog, prostate, *Acanthamoeba*, PCR, genotype T4.

INTRODUCTION

Free living amoebae of the genera *Acanthamoeba*, *Balamuthia* and *Naegleria* include several species/strains that are able to cause keratitis, encephalitis and

disseminated infections in animals and humans (Khan 2006). *Acanthamoebae* are found in diverse habitats such as water, soil or dust. Thus, it is not surprising that we often come across and interact with these organisms. Previously Chappell *et al.* (2001) showed that more than 80% of the normal human population exhibited antibodies against *Acanthamoeba*. This clearly indicated that these are one of the most ubiquitous organisms and that they often come in contact with humans and other animals.

Animal infections due these amoebae have been reported worldwide in many organisms such as horses,

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cattle and birds. Focusing on *Acanthamoeba* infections in domestic animals, it is important to mention that they have been reported as causative agents of encephalitis in three dogs (Ayers *et al.* 1972, Bauer *et al.* 1993, Brofman *et al.* 2003) and two cases of disseminated infections (Dubey *et al.* 2005, Kent *et al.* 2011).

A case of prostatitis in a mixed breed dog due to *Acanthamoeba* genotype T4 is reported in this study. To the best of our knowledge, this is the first report of prostatitis in a dog due to *Acanthamoeba* genus.

HISTORY

A ten year old mixed breed dog was brought to *Cruz de Piedra* Veterinary Clinic, La Laguna, Tenerife, Canary Islands, Spain due to a kidney dysfunction and was admitted for exploration of its organs by echography. After observation, all organs were in normal conditions with the exception of the prostate which showed signs of inflammation. Moreover, observation of the lymph nodes was compatible with inflammatory lymphadenopathy and / or an infectious process. An ultrasound-guided puncture was thus performed for further cytological and microbiological study.

CLINICAL EXAMINATION AND DIAGNOSIS

Abdominal Echography was performed at 6.6 MHz with a MyLab30Vet ultrasound system (Esaote, Barcelona, Spain) using a Micro-convex Array with 14 mm ray of curvature and able to work in a range of 9 to 3 MHz.

After performance of the ultrasound-guided puncture, the obtained fluid was observed under the microscope and culture in 2% non-nutrient agar (NNA) plates covered with heat-killed *E. coli* at 22°C and 37°C. Trophozoites were able to grow in these plates and were then prepared for their isolation and axenification in PYG medium (0.75% proteose peptone (wt/vol), 0.75% yeast extract (wt/vol), and 1.5% glucose (wt/vol), with 50 µg/ml gentamicin (Sigma) as previously described (Lorenzo-Morales *et al.* 2006). After axenification, *Acanthamoeba* trophozoites were harvested at a density of 2×10^7 parasites/ml. The cells were pelleted (500 g) for 10 min. at room temperature and washed three times with phosphate-buffered saline (PBS), pH 7.2. Cell pellets were resuspended in lysis buffer (50

mM NaCl, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0) and incubated at 55°C for 1 h with 0.25 mg/ml Proteinase K. The DNA samples were purified from amoebae isolates by the phenol-chloroform method as previously described (Lorenzo-Morales *et al.* 2006). rRNA gene amplifications (DF3 region) were performed as previously described with minimal modifications (Booton *et al.* 2002) in a 50 µl volume containing 1.25 U Taq DNA polymerase (Applied Biosystems, New Jersey), 10 ng DNA, 4 mM MgCl₂, 200 µM dNTP and 0.5 µM each primer. Amplification products were fractionated by 2% agarose electrophoresis stained with a solution of 0.5 µg/ml of ethidium bromide and visualized under UV light.

PCR products were purified using the Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced using an automated fluorescent sequencing system (Servicio de Secuenciación SEGAI, University of La Laguna). The obtained sequences were aligned using Mega 4.0 software program (Kumar *et al.* 2004). Genotype identification was based on sequence analysis of DF3 region as previously described (Lorenzo-Morales *et al.* 2006, Niyyati *et al.* 2009) by comparison to the available *Acanthamoeba* DNA sequences in Genbank or with the *Acanthamoeba* DNA database (Department of Molecular Genetics, The Ohio State University, OH, USA). The DF3 sequence for the new isolate is deposited in the GenBank database under the accession number: **JN555599**. Phylogenetic analyses were carried out using maximum parsimony, minimum evolution and maximum likelihood optimality criteria, implemented in Mega 4.0 (Kumar *et al.* 2004). Transition : transversion ratios were estimated by maximum likelihood heuristic searches. Estimates of node support were obtained by performing 500 bootstrap replicates.

The abdominal echography was carried out in order to check the status of the bladder, kidneys, spleen, liver, adrenal glands, iliac lymph node and prostate in the dog that was previously diagnosed with a kidney dysfunction. The kidneys, liver, spleen and adrenal glands were showing a normal echotexture and echogenicity. The bladder was normo-relaxed with the presence of large amounts of urine sediment with an homogenous and isoechoic wall. Regarding the prostate (size 4.37 × 4.58 cm), the parenchyma was mostly heterogeneous with the presence of a multiple hyperechoic dotted pattern with various anechoic foci with non-defined margins. The larger focus was approximately 1.18 × 2.38 cm in size. Moreover, the iliac lymph node was showing an increase in size (0.66 × 3.16 cm) with a hypere-

choic and rounded morphology (Fig. 1). The obtained results in this echography concluded that the prostate was showing signs of inflammation. Moreover, observation of the lymph nodes was compatible with inflammatory lymphadenopathy and/or an infectious process. Thus, an ultrasound-guided puncture was performed for further cytological and microbiological study.

When the extracted fluid from the animal was examined for any pathogen under light microscopy, a large amount of *Acanthamoeba* trophozoites were observed in this purulent fluid (Fig. 2). No other bacterial or viral pathogens were detected in this fluid. Therefore, drops of this fluid were cultured in 2% NNA plates for the isolation and axenification of the amoebae. After this process, DNA was extracted directly from the fluid and from the amoebae cultures (plates and axenified ones) and DF3 fragment PCR was carried out in order to verify the microscopy observations. PCR was positive in all samples and after purification (Fig. 3), the obtained sequence allowed the classification of this isolate into *Acanthamoeba* genotype T4 after phylogenetical analysis (Fig. 4). Unfortunately at this stage, the dog's owner decided to reject any kind of treatment or therapy against acanthamoebae and also any further analyses of the status of the animal.

DISCUSSION

Acanthamoeba genotype T4, a pathogenic one, is widely spread in the environment and it is also the most common genotype in human infections cases (Khan 2006). Therefore, the source of infection in this case was not identified. The route of entry has been speculated to be oral or nasal since an haematogenous spread was suspected.

Most of the previously reported cases of infections in dogs (Ayers *et al.* 1972, Bauer *et al.* 1993, Brofman *et al.* 2003, Dubey *et al.* 2005, Kent *et al.* 2011) were manifesting as encephalitis processes or multisystemic disseminated infections. In the case reported in this study, even when the diagnosis was relatively quick, only the prostate appeared to be affected by acanthamoebae. However and due to the lack of response of the dog's owner it was not possible to carry out anymore analyses in the dog and thus, it is impossible to confirm whether only the prostate was affected. Nevertheless, if the abdominal echography data are considered, all the studied organs were in normal conditions with the exception



Fig. 1. Echography of the prostate showing alteration of the prostatic parenchyma, with various irregular anechoic foci and multiple hyperechoic dotted pattern with non-defined margins.

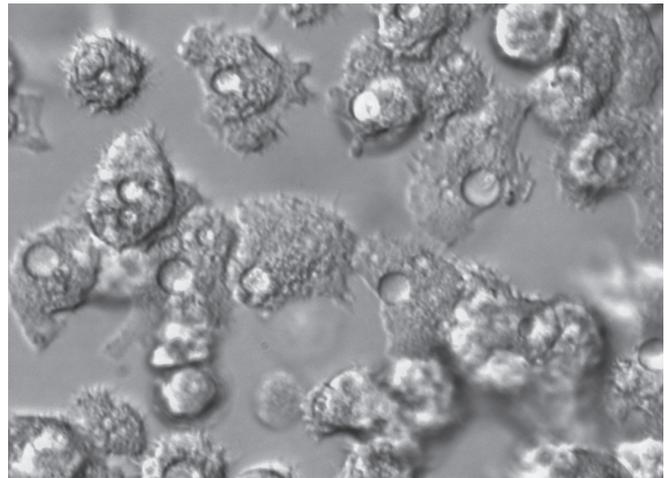


Fig. 2. *Acanthamoeba* trophozoites and cysts in the fluid extracted from the prostate.

of the prostate. Further data such as the immunological status of the animal could have helped in a better description or elucidation of the infectious process that this animal was suffering. Based on the presented data, a prostatitis due to *Acanthamoeba* genotype T4 was reported.

To the best of our knowledge, this is the first reported case of prostatitis caused by acanthamoebae in

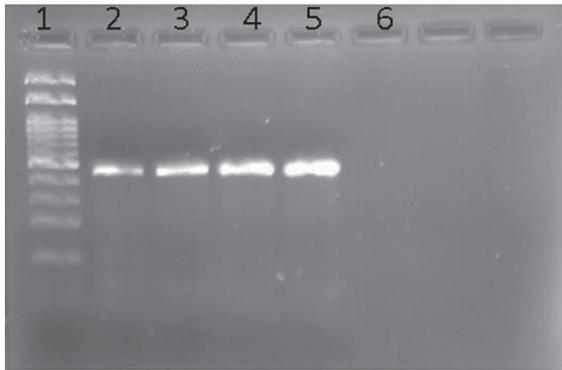


Fig. 3. *Acanthamoeba* specific PCR using JDP1/JDP2 primers. Lane 1: Molecular Weight Marker 100bp ladder; Lane 2: Prostate Fluid; Lane 3: Non-Nutrient Agar Amoebic Culture; Lane 4: Axenic culture; Lane 5: Positive control *Acanthamoeba castellanii* Neff ATCC 30010 DNA; Lane 6: Negative control, bidistilled water.

a dog. Even when it seems that these infections are uncommon in dogs, the reported case together with the previously described ones should raise awareness within veterinary professionals of the possibility of amoebic infections in domestic animals.

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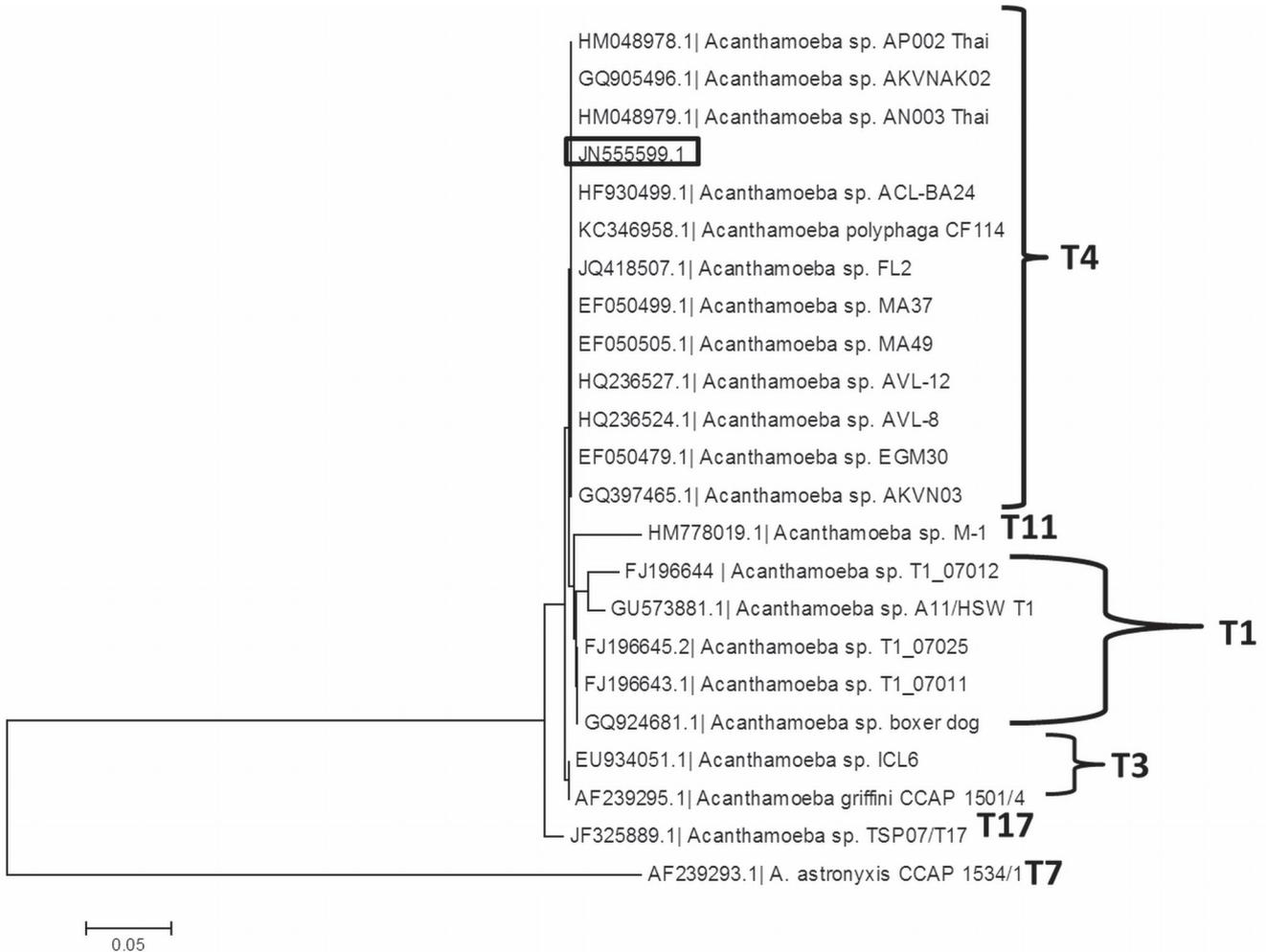


Fig. 4. Phylogenetical analysis of the DF3 sequence of the *Acanthamoeba* strain isolated from the prostate revealed that the strain belonged to genotype T4.

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