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Comparative analysis of *Tritrichomonas foetus* (Riedmüller, 1928) cat genotype, *T. foetus* (Riedmüller, 1928) cattle genotype and *Tritrichomonas suis* (Davaine, 1875) at 10 DNA loci ☆

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ABSTRACT

The parasitic protists in the genus *Tritrichomonas* cause significant disease in domestic cattle and cats. To assess the genetic diversity of feline and bovine isolates of *Tritrichomonas foetus* (Riedmüller, 1928) Wenrich and Emmerson, 1933, we used 10 different genetic regions, namely the protein coding genes of cysteine proteases 1, 2 and 4–9 (CP1, 2, 4–9) involved in the pathogenesis of the disease caused by the parasite. The cytosolic malate dehydrogenase 1 (MDH1) and internal transcribed spacer region 2 of the rDNA unit (ITS2) were included as additional markers. The gene sequences were compared with those of *Tritrichomonas suis* (Davaine, 1875) Morgan and Hawkins, 1948 and *Tritrichomonas mobilensis* Culberson et al., 1986. The study revealed 100% identity for all 10 genes among all feline isolates (=*T. foetus* cat genotype), 100% identity among all bovine isolates (=*T. foetus* cattle genotype) and a genetic distinctness of 1% between the cat and cattle genotypes of *T. foetus*. The cattle genotype of *T. foetus* was 100% identical to *T. suis* at nine loci (CP1, 2, 4–8, ITS2, MDH1). At CP9, three out of four *T. suis* isolates were identical to the *T. foetus* cattle genotype, while the *T. suis* isolate SUI-H3B sequence contained a single unique nucleotide substitution. *Tritrichomonas mobilensis* was 0.4% and 0.7% distinct from the cat and cattle genotypes of *T. foetus*, respectively. The genetic differences resulted in amino acid changes in the CP genes, most pronouncedly in CP2, potentially providing a platform for elucidation of genotype-specific host-pathogen interactions of *T. foetus*. On the basis of this data we judge *T. suis* and *T. foetus* to be subjective synonyms. For the first time, on objective nomenclatural grounds, the authority of *T. suis* is given to Davaine, 1875, rather than the commonly cited Gruby and Delafond, 1843. To maintain prevailing usage of *T. foetus*, we are suppressing the senior synomym *T. suis* Davaine, 1875 according to Article 23.9, because it has never been used as a valid name after 1899 and *T. foetus* is widely discussed as the cause of bovine trichomonosis. Thus bovine, feline and porcine isolates should all be given the name *T. foetus*. This promotes the stability of *T. foetus* for the veterinary and economically significant venereal parasite causing bovine trichomonosis.

1. Introduction

Flagellated protists in the genus *Tritrichomonas* Kofoid, 1920 are traditionally considered as mucus-dwelling parasites characterised by three anterior flagella (BonDurant and Honigberg, 1994). The best-studied member of the group is *Tritrichomonas foetus* (Riedmüller, 1928), a parasite of cattle. This parasite causes a sexually transmitted disease in cattle that can result in early embryonic death, infertility and abortion, even permanent sterility, which causes significant economic losses worldwide (Yule et al., 1989; BonDurant, 1997). Artificial insemination has successfully been used to control the disease, but in many parts of the world the parasite is still prevalent (Campero and Gottstein, 2007). In countries that have successfully eradicated the parasite, strict regulations to prevent a reintroduction are in place. The disease, termed bovine trichomonosis, remains notifiable in many countries around the world (including Australia, USA, Argentina, Switzerland, New Zealand and the Czech Republic).

☆ Nucleotide sequence data reported in this paper are available in GenBank under Accession numbers: JX187000–JX187133 and JX648146–JX648175.

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Recently, *T. foetus* was recognised as the causative agent of feline enteric trichomonosis (Levy et al., 2003; Gookin et al., 2004; Hale et al., 2009; Bell et al., 2010). In contrast to the bovine disease, infection in cats manifests as large-bowel disease with signs varying from asymptomatic carriage to intractable, chronic diarrhoea which may be continuous, or more commonly, follow a waxing and waning time course (Gookin et al., 2001; Foster et al., 2004). Feline trichomonosis is prevalent in typically young, densely housed populations of both pure and mixed breeds, with no sex predilection reported (Holliday et al., 2009; Stockdale et al., 2009). The disease has been recognised worldwide with the prevalence of *T. foetus* estimated to be as high as 30% (reviewed in Frey and Müller, 2012). The increased incidence of infection in multi-cat environments is believed to be due to the reliance on close, if not direct, contact between individuals (Gookin et al., 2004). *Tritrichomonas foetus* can survive up to 7 days in cat faeces, suggesting that grooming and faecal contamination of the environment may play an equal role in the epidemiology of feline enteric trichomonosis, especially in the multi-cat environment (Hale et al., 2009; Van der Saag et al., 2011). A treatment for feline enteric trichomonosis is available, i.e. ronidazole (Gookin et al., 2006; LeVine et al., 2011). However, the practise of treating only clinically affected animals, coupled with a lack of knowledge on disease epidemiology, has negative consequences for mitigating the spread of the pathogen. Despite the improvement in diagnosis and management of trichomonosis, little has been learned about the pathogenesis of the disease in cats.

At the cellular level, results by Stockdale et al. (2007, 2008) clearly demonstrate that significant differences exist in both infectivity and pathogenicity for feline *T. foetus* and bovine *T. foetus* isolates in experimentally infected cats and cattle. These combined results indicate that there are key differences between isolates influencing parasite-host species range. Recent reports show small but consistently detectable genetic differences between the cattle and cat isolates, suggesting the presence of two host-adapted genotypes of *T. foetus* (Šlapeta et al., 2010; Reinmann et al., 2012; Sun et al., 2012). The nucleotide and amino acid differences in the cysteine protease 8 between the cattle and cat genotypes of *T. foetus* offered the first insight into possible adaptation to its preferred host (Sun et al., 2012).

Soon after bovine trichomonosis was recognised and *T. foetus* named as the agent causing the disease, parasitologists debated the apparent similarity of *T. foetus* with the porcine trichomonad – *Tritrichomonas suis* – isolated from the nasal cavity, caecum and stomach of pigs. In fact the apparent similarity between *T. foetus* and *T. suis* became one of the parasitological enigmas. Hibler et al. (1960) reviewed the pig trichomonads isolated from nasal cavities and caeca of pigs, and concluded that if tritrichomonads from pigs and cattle were to be placed in a single species, the name *T. suis* would have priority over *T. foetus*. Levine (1973) stated that "... it might still be worthwhile to retain both names simply as matter of convenience" and Honigberg (1978) concluded that "... because much confusion would be created among parasitologists and veterinarians if the taxonomic status of especially *T. foetus* were changed, it seems advisable to postpone the implementation of such changes". However, none of these and more recent articles suggested any practical solution based on objective data (Tachezy et al., 2002; Lun et al., 2005; Frey and Müller, 2012).

The aim of this study was to use an array of protein coding genes to establish genetic conservation of the cattle and cat genotypes of *T. foetus* (Riedmüller, 1928) and *T. suis* (Davaine, 1875) from pigs, in particular, using the multigene cysteine proteases. To evaluate the genetic diversity these genotypes were compared with the most closely related taxon, *Tritrichomonas mobilensis* Culberson et al., 1986, from squirrel monkeys. Using 10 independent genetic loci, we confirmed genetic distinctness of the cattle and cat genotypes of *T. foetus*, and demonstrated identity of the cattle genotype of *T. foetus* with *T. suis*, which has prompted us to re-evaluate the nomenclature and taxonomy of the latter species.

2. Material and methods

2.1. Feline and bovine isolates of T. foetus, T. suis and T. mobilensis

Twenty isolates of *Tritrichomonas* spp. were obtained for this study (Table 1). Seven feline *T. foetus* isolates were included in this study (Hale et al., 2009; Šlapeta et al., 2010; Reinmann et al., 2012; Sun et al., 2012). Eight bovine *T. foetus* isolates were included in this study: one Australian reference strain YVL-W (Department of Agriculture, Fisheries and Forestry, Queensland), one reference strain from the Czech Republic (Department of Parasitology, Charles University, Prague), and six Argentinean isolates (Tachezy et al., 2002; McMillen and Lew, 2006; Reinmann et al., 2012). Four porcine *T. suis* were used to investigate their relationship to the bovine and feline isolates (Tachezy et al., 2002). In addition *T. mobilensis* was used in this study (Culberson et al., 1986). DNA dissolved in water was stored at −20 °C prior to amplification.

2.2. Primer design and PCR amplification

Primers were designed for the *Tritrichomonas* cysteine proteases (CP1, 2, 4–9) and cytosolic malate dehydrogenase 1 (MDH1) using sequences available from GenBank (Table 2). Primers were designed using CLC Main Workbench 6.2 (CLC bio, Denmark). Internal transcribed spacer (ITS) rDNA was amplified by PCR using primers TFR3 (5′-CGG GTC TTC CTA TAT GAG ACA GAA CC-3′) and TFR4 (5′-CCT GCC GTT GGA TCA GTT TCG TTA A-3′) according to Felleisen et al. (1998) and produced a 348 bp amplicon. All primers were synthesized by Macrogen Ltd. (Seoul, Korea).

All PCR amplifications were performed with MyTaq™ Red Mix (BioLine, Australia). Primers were added at a concentration of 0.25 μM each. The PCR was run using the following cycling conditions: 95 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s for 35 cycles (40 cycles resulted in no difference in sequencing outcome, data not shown). All reactions were initiated at 95 °C for 5 min and concluded at 72 °C for 5 min. PCRs were amplified in the Eppendorf Mastercycler Personel or Eppendorf Mastercycler Gradient. Each PCR mix (40 μl) contained 2 μl of the sample DNA. All PCRs were run with negative controls (distilled water). Resulting products were resolved in 2% (w/v) agarose. All PCRs yielded unambiguous single bands of expected sizes (see Table 2). CP9 was further amplified using the MyTaq™ HS Mix (BioLine) and KAPA2G™ Fast HotStart ReadyMix (KAPA BioSystems, MA, USA). All sequences were directly and bidirectionally sequenced using amplification primers at Macrogen Ltd. Sequences were assembled, aligned with related sequences and analysed using CLC Main Workbench 6.2 and deposited in GenBank (National Center for Biotechnology Information, NCBI) under the Accession Numbers: JX187000–JX187133, JX648146–JX648175. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011). Multiple sequences alignments were appended and absent gene sequences coded as missing data. A phylogenetic tree was inferred using maximum likelihood and the bootstrap support inferred from 100 replicates.

3. Results

3.1. Tritrichomonas foetus cat genotype is distinct from T. foetus cattle genotype at 10 different loci

All 10 gene loci were successfully amplified using DNA of *T. foetus* from cats and *T. foetus* from cattle. PCR amplicons were