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Tritrichomonas foetus from domestic cats and cattle are genetically distinct

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ABSTRACT

The genetic relationship amongst *Tritrichomonas foetus* isolated from domestic cats and cattle was investigated by DNA sequencing of the internal transcribed region of the ribosomal DNA unit and the TR7/TR8 variable-length repeat. The results reject the hypothesis that *T. foetus* from domestic cats is genetically identical to *T. foetus* in cattle. We suggest recognition of a 'cat genotype' and a 'cattle genotype' of *T. foetus*. Review of public nucleotide repositories revealed that the 'cat genotype' has not been isolated from cattle nor the 'cattle genotype' recovered from cats. However, at least one cat isolate has been shown to induce disease in experimentally infected cattle. We conclude that these genotypes fall within the single species *T. foetus*.

1. Introduction

Tritrichomonas foetus is a flagellated protozoan parasite recognised as a primary agent causing large bowel diarrhoea in cats (Levy et al., 2003). It has been assumed to be the same organism which causes venereal disease and abortion storms in naturally bred cattle and is a commensal in the nasal cavity and caecum of pigs (BonDurant, 1997). However, the source of *T. foetus* and its epidemiology in feline populations remains poorly characterised. Current evidence does not support acquisition of *T. foetus* from cattle or pigs, dietary sources or water (Gookin et al., 2004). Nevertheless, viable trophozoites of *T. foetus* in cat faeces survive for extensive period of time, suggesting that grooming and faecal-oral transfer may play a role in establishing *T. foetus* infection in multi-cat environments (Hale et al., 2009).

Experimental studies have demonstrated that a cat isolate of *T. foetus* can infect cattle and conversely a cattle isolate of *T. foetus* can infect cats. Disease in cattle caused by the cat isolate AUTf-1 of *T. foetus* is comparable, but not identical, to that caused by the cattle isolate D-1 of *T. foetus* (Stockdale et al., 2007). There were two remarkable differences; firstly, there was significant difference in the histopathological appearance of the columnar epithelium of the uterine surface which was intact in heifers inoculated with the cat isolate, while in the cattle isolate group it was infiltrated with inflammatory cells and characterised by the disappearance of the uterine glands (Stockdale et al., 2007). Secondly, all eight heifers cleared the cattle isolate of *T. foetus* during the 20 weeks of the experiment, compared to only two out of eight heifers infected with the cat isolate of *T. foetus* were culture negative at 20 week post inoculation (Stockdale et al., 2007). Using the same isolate, Stockdale et al. (2008) demonstrated that one of six cats inoculated with D-1 isolate of *T. foetus* established infection of the intestinal tract. This cat was culture positive on day 32 post inoculation and at necropsy (35 days post inoculation) and a burden of 1.75×10^4 *T. foetus* organisms/ml was detected in the cat's caecum (Stockdale et al., 2008). These results suggest some phenotypic differences in pathology and persistence between cat and cattle isolates, and they were interpreted to be beyond normal intra-specific variation (Stockdale et al., 2008).

The aim of this study was to investigate the genetic identity of *T. foetus* from domestic cats and cattle using newly isolated domestic cat isolates and resolve the hypothesis that these organisms are genetically identical to isolates from cattle. We reject this hypothesis, because our results support species specific lines of infections of hosts. We suggest recognition of a 'cat genotype' and a 'cattle genotype' of *T. foetus*.

2. Materials and methods

2.1. Cat isolates of *Tritrichomonas foetus*

2.1.1. Cat 1

A 14-month-old Abyssinian intact female cat was presented with on-going diarrhoea. Faecal smears did not reveal the presence of flagellates. In-Pouch TF-feline (In-Pouch; TropBio, Townsville,

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QLD) under license from BioMed Diagnostics (White City, OR, USA) was inoculated with a rectal swab and incubated at 37 °C. Motile flagellates were observed in the pouch 24 h post inoculation. The culture was subcultured into a new In-Pouch and maintained in Modified Diamonds Medium (MDM; ATCC medium 719) by subculturing. The axenised isolate designated Sydney08/1 was cryopreserved in liquid nitrogen using 10% dimethyl sulfoxide, it is the same isolate studied and referred to as G08/1 by Hale et al. (2009).

The large bowel diarrhoea of the Cat 1 was first apparent when the kitten was 4 months old. At that time, large number of unidentified flagellates was observed on direct faecal smear. Over the next 10 month, the diarrhoea persisted, although waxing and waning in severity. The cat has been treated initially with toltrazuril, then with metronidazole and subsequently with fenbendazole. Faecal samples were consistently negative for *Giardia* and *Cryptosporidium*. The cat ate well and had not vomited. The cat was domiciled in a breeding cattery with 15–20 other cats of varying ages in urban greater Sydney area, New South Wales, Australia. None of the other in contact cats had diarrhoea, although the other two kittens in her litter had self resolving diarrhoea at 4 months of age. Cat 1 was isolated and treated for 14 days with ronidazole (orally once a day, 30 mg kg^{-1}, BOVA Compounding Pharmacy, Caringbah, NSW 2229), and was In-Pouch culture negative 7 days after treatment. However, a culture 14 days post the ronidazole treatment was positive for *T. foetus*. Although Cat 1 was isolated for 4 weeks, but put together with a sire following this time.

2.1.2. Cat 2

A 7-month-old Russian Blue intact female cat was presented with chronic large bowel diarrhoea. Motile flagellates were observed on a direct smear. The In-Pouch was inoculated with a rectal swab and incubated at 37 °C. Motile flagellates were observed in the pouch 24 h post inoculation. The culture was subcultured, axenised and cryopreserved in liquid nitrogen. The isolate was designated Sydney08/2.

The large bowel diarrhoea first developed at 2 month-of-age, soon after the cat was acquired from the breeder. At 3 month-of-age, diarrhoea containing fresh blood and tenesmus was observed. No vomiting was noted and the cat was eating well. Faecal samples were negative for *Giardia* and *Cryptosporidium*. The cat was initially treated with toltrazuril due to suspected acute cystoisosporosis, however, she continued to have intermittent diarrhoea. Subsequently, Hill's feline i/d diet and potentiated sulphonamides were prescribed. At 4 month, pronounced bloody diarrhoea lasting 3 days was observed; *Giardia* was identified and a 14 days of metronidazole was prescribed; however, intermittent diarrhoea continued. At 5 month, metronidazole was repeated with no discernable improvement. Faecal samples were negative for *Giardia* and *Cryptosporidium*.

At the time of culture the cat was housed with an intact male Russian Blue in greater Sydney; there were no other pets in the household. The male was acquired 2 month prior to the female. The male had bloody diarrhoea when he first arrived, which settled. Then when female arrived she developed similar clinical signs and he developed bloody diarrhoea again. Both cats exhibited identical clinical signs and were treated for 14 days with ronidazole (orally once a day, 30 mg kg^{-1}, BOVA Compounding Pharmacy, Caringbah, NSW 2229). No additional data is available regarding the outcome of this treatment. Her diarrhoea resolved but then bloody diarrhoea recurred again 4 months later – ronidazole was repeated and she has been fine since. He has had on-going bloody diarrhoea, although did improve after initial course. He has never tested positive on pouch culture, but was not tested before initial treatment with ronidazole.

2.1.3. Cat 3

A cat isolate of *T. foetus* was cultured from a clinical sample using an In-Pouch inoculated with a rectal swab and incubated at 37 °C. Motile flagellates were observed in the pouch 48 h post inoculation, although the culture was overgrown by a rapidly growing yeast and died. Approximately 200 μl of the In-Pouch medium with motile flagellates was used for DNA isolation (Sydney09/1).

2.1.4. Cat 4

A 12-week-old male desexed Tonkinese kitten was presented with on-going diarrhoea with blood and mucus, a very bloated abdomen and pink moist mucus membranes. Motile flagellates were observed on a direct faecal smear. The In-Pouch was inoculated with a rectal swab and incubated at 37 °C. Motile flagellates were observed in the pouch 24 h post inoculation. The culture was subcultured, axenised and cryopreserved in liquid nitrogen. The isolate was designated Sydney10/1.

The diarrhoea of the Cat 4 was apparent at the moment the kitten was brought from the breeder. After 2 weeks the diarrhoea got significantly worse, with a lot of fresh blood and mucus present and very liquid faeces. The cat has been treated initially with toltrazuril, that did not resolve the diarrhoea. Cat 4 was treated for 14 days with ronidazole (orally once a day, 30 mg kg^{-1}, BOVA Compounding Pharmacy, Caringbah, NSW 2229), and was In-Pouch culture negative 7 days after treatment, faeces were normal and the bloated abdomen has resolved.

2.2. Cattle Tritrichomonas foetus DNA

DNA from cattle *T. foetus* reference strains from Australia (DPI-QLD) and the Czech Republic (CZ) was resourced for this study. An Australian strain YVL-W (McMillen and Lew, 2006) was kindly provided by Dr. Ala E. Lew (Animal Research Institute, Department of Primary Industries and Fisheries, Queensland). A strain KVc-1 (Tachezy et al., 2002) was kindly provided by Dr. Pavel Doležal (Department of Parasitology, Charles University Prague, Czech Republic).

2.3. DNA isolation and PCR amplification

DNA from the cat isolates was isolated using PureLink Genomic DNA Kit (Invitrogen, Australia) according to the manufacturer's instructions. DNA was eluted into two consecutive aliquots of 100 μl distilled water and stored at −20 °C prior to amplification.

Internal transcribed spacer rDNA (ITS) was amplified independently using two sets of primers. Oligonucleotides TFR1 (5′-TGC TTC AGT TCA GCG GGT CTT CC-3′) and TFR2 (5′-CGG TAG GTG AAC CTG CCG TTG G-3′) were used as primers in the polymerase chain reaction (PCR) according to Felleisen (1997). Oligonucleotides 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′) were used as primers in PCR according to Felleisen et al. (1998). For amplification using the TFR1/TFR2 region we used 2× MasterMix (Fermentas, USA) and for amplification using the TFR3/TFR4 region we used 2× EconoTaq PLUS *GREEN* MasterMix (Lucigen, USA). Both mastermixes contain Taq DNA polymerase and were used according to the manufacturer's instructions. Primers were added at 0.25 μM concentration each. The PCR was run for 40 cycles (95 °C for 0.5 min, 66 °C for 0.5 min, 72 °C for 1 min). Both reactions were initiated at 95 °C for 5 min and concluded at 72 °C for 7 min. Both PCRs were amplified in an Eppendorf Mastercycler Personel. Each PCR mix (25 μl) contained 2 μl of the sample DNA. All PCRs were run with negative controls (distilled water). Resulting products were resolved in 1.5% (w/v) agarose. Positive bands of expected sizes, TFR1/TFR2 region 372 nt and TFR3/TFR4 region 348 nt, were bidi-