Veterinary Parasitology 212 (2015) 105–110

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

High prevalence of *Tritrichomonas foetus* 'bovine genotype' in faecal samples from domestic pigs at a farm where bovine trichomonosis has not been reported for over 30 years

Kai Mueller[1], Victoria Morin-Adeline[1], Katrina Gilchrist, Graeme Brown, Jan Šlapeta*

Faculty of Veterinary Science, McMaster Building B14, University of Sydney, New South Wales 2006, Australia

ARTICLE INFO

Article history:
Received 4 May 2015
Received in revised form 11 August 2015
Accepted 12 August 2015

Keywords:
Trichomonosis
Cow
Pig
Cat
Cysteine protease
Genotyping

ABSTRACT

Bovine venereal trichomonosis caused by the flagellate *Tritrichomonas foetus* is a notifiable disease in Australia. While, *T. foetus* is pathogenic in both cattle and cats, it has long been established that the same *T. foetus* colonises the stomach, caecum and nasal cavity of pigs without apparent clinical significance. Multi-locus genotyping grouped the non-pathogenic porcine *T. foetus* with the pathogenic 'bovine genotype', rather than with the 'feline genotype' *T. foetus.* Bovine trichomonosis is now uncommon due to wide-spread use of artificial insemination, however, whether *T. foetus* remains prevalent in pigs where bovine trichomonosis has been eradicated remains unknown. We surveyed faecal samples from pigs farmed in close proximity with *T. foetus*-negative cattle. The Modified Diamond's Medium assay used were 77.4% (24/31) positive for trichomonads and 64.50% (20/31) were *T. foetus*-positive based on real-time PCR and conventional PCR. An axenic reference strain of *T. foetus*, designated PIG30/1 was established. In addition, a novel trichomonad ITS rDNA, PIG12, closely related to sequences from *Trichomitus* spp is reported. Multi-locus genotyping at nine loci matched PIG30/1 to the 'bovine genotype' *T. foetus*. In conclusion, cross-species transmission of *T. foetus* between pigs and cows from environmental exposure of *T. foetus*-contaminated pig faeces is unlikely. Domestic *T. foetus*-positive pigs possess a negligible risk of a successful *T. foetus* transmission event to cattle.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Tritrichomonas foetus is a flagellate protozoan parasite of the bovine reproductive tract that causes bovine trichomonosis (BonDurant, 1997; Campero and Gottstein, 2007). The parasite is exclusively transmitted by mating with asymptomatic *T. foetus* infected bulls as the only source of infection (Campero and Gottstein, 2007). Past epidemiological studies have not demonstrated involvement of any reservoir or transport host species. It was long known, however, that the same *T. foetus* is also found in the stomach, caecum and nasal cavity of pigs without apparent clinical significance (Hammond et al., 1958; Hibler et al., 1960; Mostegl et al., 2011; Pakandl, 1994). More recently, *T. foetus* is recognised as the cause of chronic large bowel diarrhoea in domestic cats worldwide (Bell et al., 2010; Levy et al., 2003; Lim et al., 2010). Multilocus genotyping confirmed that the cattle and pig isolates represent the 'bovine genotype' of *T. foetus* while the cat isolates represent a closely related 'feline genotype' of *T. foetus* (Morin-Adeline et al., 2014; Morin-Adeline et al., 2015; Šlapeta et al., 2012).

Bovine trichomonosis is now uncommon in many parts of the world due to the widespread use of artificial insemination using strictly monitored *T. foetus*-free bulls (BonDurant, 1997). Whether, *T. foetus* remains prevalent in pigs in places where bovine trichomonosis has been eradicated remains unknown. Porcine *T. foetus* was experimentally demonstrated to be capable of producing disease in cows and may therefore, pose a theoretical opportunity for the re-introduction of *T. foetus* into cattle (Fitzgerald et al., 1958). On the other hand, a high prevalence of *T. foetus* in pigs within the vicinity of *T. foetus*-free cattle would confirm different isolate-specific epidemiologies and therefore, a negligible risk of porcine *T. foetus* transmission to cattle.

Diagnosis of *T. foetus* in pigs is not considered by veterinarians. There is no accepted gold standard for its detection in pigs, however, microscopic examination following culture and PCR-based assays are two commonly used methods for the detection of *T. foetus* in cattle (Campero et al., 2003; Guerra et al., 2014; Mukhufhi et al., 2003). Various studies have reported similar sensitivities for

* Corresponding author.
E-mail address: jan.slapeta@sydney.edu.au (J. Šlapeta).
[1] Contributed equally

http://dx.doi.org/10.1016/j.vetpar.2015.08.010
0304-4017/© 2015 Elsevier B.V. All rights reserved.

both culture (67.7%–100%) and PCR-based (65.9%–90.0%) assays, however, detection via culture has the added advantage of yielding living parasites that can be preserved for use as a reference strain (Hale et al., 2009; Parker et al., 2003; Parker et al., 1999). The culture of trichomonads from pigs has been previously performed using various media such as liver broth infusion, diphasic media and the commercially available InPouchTF culture systems (Dimasuay and Rivera, 2013; Hibler et al., 1960; Solaymani-Mohammadi et al., 2004). The in-house Modified Diamond's Media (MDM) is recommended by the World Organisation for Animal Health (OIE) and is widely used for the culture of trichomonads (http://www.oie.int/). Modified Diamond's Media (MDM) offers a high diagnostic sensitivity (100%) for the detection of *T. foetus* in cat faeces whilst also sustaining the highest peak concentrations of *T. foetus* compared to other media (Hale et al., 2009; Lun et al., 2000).

The aim of this study was to demonstrate the presence of *T. foetus* in Australian domestic pigs and their comparison to other strains of *T. foetus*. To achieve this, the MDM-based culture technique was validated using *T. foetus*-spiked faecal samples. The validated assay was used to confirm the presence of trichomonads in Australian pigs. Subsequently, conventional and real-time PCR assays were used for species-level confirmation. Multilocus genotyping of the new axenic pig isolate of *T. foetus* confirms its 'bovine genotype' status. We demonstrate that the 'bovine genotype' of *T. foetus* is common in domestic pigs and viable parasites can be isolated from fresh pig faeces at a location where bovine trichomonosis has not been reported for over 30 years.

2. Material and methods

2.1. Trichomonad culture and assay validation

Modified Diamond's Medium (MDM) (200 ml) prepared with 4 g of Trypticase peptone (BD211921; Becton, Dickinson and company, NSW, Australia), 2 g yeast extract (BD211929; Becton, Dickinson and company, NSW, Australia) and 1 g maltose (BD216830; Becton, Dickinson and company, NSW, Australia) at pH 7.2 (ATCC Medium 719 without agar; http://www.atcc.org/) and supplemented with 10% inactivated sheep serum (Life Sciences, Australia) was used throughout (Hale et al., 2009). For maintenance of cultures, the antibiotics PenStrep & Fungizone were used at a final concentration of 10 U/ml penicillin, 10 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Gibco 15240062, Life Sciences, Australia) (Hale et al., 2009; Van der Saag et al., 2011). All cultures were maintained in sterile 15 ml glass bijou bottles and sub-cultured daily. An axenic feline isolate of *T. foetus* G10/1 isolated from a 12-week-old male desexed Tonkinese kitten was used as a positive control throughout the study (Šlapeta et al., 2010; Šlapeta et al., 2012).

Mid-exponential phase trophozoites of axenically cultured *T. foetus* G10/1 'feline genotype' were washed twice in sterile phosphate-buffered saline (PBS, pH 7.2) and counted using the Neubauer Improved counting chamber. Serial dilutions of $2 \times 10^{2-4}$ of G10/1 *T. foetus* per g of spiked pig faeces were used for culture detection limits. Prior to faecal spiking, pig faeces were collected from May Farm (Camden Farms, University of Sydney), stored at 4 °C for 10 days and confirmed trichomonas and *T. foetus*-negative. In less than 30 min, all pig faecal samples were spiked with the appropriate *T. foetus* concentration in fresh MDM supplemented with 1000 U/ml penicillin (P-3032; Sigma–Aldrich, Australia) and 15 mg/ml streptomycin (S-9137; Sigma–Aldrich, Australia). Three replicate subsamples of approximately 20 mg of *T. foetus* G10/1-spiked pig faeces were inoculated into 5 ml of MDM. As a positive control, 2×10^2 *T. foetus* G10/1 trophozoites in PBS were inoculated into 5 ml of MDM. As a negative control, approximately 20 mg of faeces diluted 1:1 with PBS was inoculated into 5 ml MDM. All cultures were incubated at 37 °C and aliquots visually assessed at 24, 48 and 72 h post-incubation (HPI) using an Olympus BX41 microscope (Olympus, Australia). Cultures positive for *T. foetus* G10/1 were identified based on observation of the characteristic morphology and jerky movement of viable trophozoites.

2.2. Detecting trichomonads from fresh pig faecal samples

Faecal samples ($n = 31$) were collected from 10 week-old group-housed grower domestic pigs (45 pigs) at May Farm (Camden Farms, University of Sydney). Faeces were collected from pigs immediately after defecation and approximately 20 mg was individually inoculated directly into culture bottles containing 5 ml of freshly prepared MDM supplmented with 1000 U/ml penicillin and 15 mg/ml streptomycin (Sigma–Aldrich, Australia) using sterile inoculation loops (3 September 2014). The culture bottles were transported (<3 h) to the laboratory at 20–25 °C and then incubated at 37 °C. Media inoculated with 20 mg of a 6 week old, *T. foetus*-negative pig faeces kept at 4 °C that was spiked with a concentration of 2×10^3 and 2×10^2 G10/1 *T. foetus* per g served as a positive control. The negative control consisted of duplicate culture bottles containing MDM without the addition of *T. foetus*. All cultures were microscopically analysed after 24 and 48 h. A trichomonad-positive faecal sample from a farmed pig was axenised through continual passages in MDM containing the antibiotics PenStrep & Fungizone; 15 U/ml penicillin, 15 μg/ml streptomycin and 0.375 μg/ml amphotericin B (Gibco 15240062, Life Sciences, Australia) and designated as *T. foetus* PIG30/1. The axenic culture of PIG30/1 was crypreserved with 10% (v/v) dimethyl sulfoxide (DMSO, Sigma, Australia) and deposited in liquid nitrogen at the parasitology collection (Faculty of Veterinary Science, University of Sydney).

2.3. DNA isolation

Approximately 1 ml of 48 h old faecal culture with and without motile flagellates was pelleted and stored at −20 °C prior to DNA isolation. Individual frozen pellets were re-suspended in 100 μl of sterile phosphate-buffered saline (PBS, pH 7.2) and DNA was isolated using the BioLine Isolate Fecal DNA kit (BioLine, Australia) according to the manufacturer's instructions, with the omission of β-mercaptoethanol. Homogenization of *T. foetus* parasites prior to extraction was carried out in a FastPrep®-24 high-speed homogeniser (MP Biomedicals, USA) at 6 m/s for 30 s. Sterile PBS was employed as negative control. DNA was extracted from 10^5 mid-exponential phase trophozoites from an axenic PIG30/1 isolate as described above. All DNA was stored at −20 °C prior to amplification.

2.4. Real-time PCR for Tritrichomonas foetus

Diagnostic real-time PCR was based on primers TFR3 [S0001] (5′-CGG GTC TTC CTA TAT GAG ACA GAA CC-3′) and TFR4 [S0002] (5′-CCT GCC GTT GGA TCA GTT TCG TTA A-3′) specific for *T. foetus* that amplify 348 nt region of the internal transcribed spacer (ITS) rDNA (Felleisen et al., 1998; Šlapeta et al., 2010). All real-time PCR reactions used KAPA SYBR® FAST qPCR (2×) Master Mix (Kapa Biosystems, Inc., MA, USA) on CFX96 Touch™ Real-Time PCR Detection System with the corresponding CFX Manager 3.0 software (BioRad, Australia). Each real-time PCR assay was prepared to contain 200 nM of each primer and 4 μl of template DNA in a total volume of 20 μl. The annealing temperature of the real-time PCR was initially optimised based on a standard curve and efficiency using 55–65 °C gradients. All *T. foetus* real-time PCR reactions were performed in duplicate using the following cycling conditions: 3 min at 95 °C, followed by 40 cycles of 3 s at 95 °C and 20 s at 63 °C. A melt curve analysis was completed for each real-time

Download English Version:

https://daneshyari.com/en/article/5802337

Download Persian Version:

https://daneshyari.com/article/5802337

Daneshyari.com