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Original article

Detection of *Pentatrichomonas hominis* in dogs using real-time PCR

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Abstract

Trichomonadidae family is a protozoan occurring in different animal species. It inhabits the gastrointestinal and urinary tracts. *P. hominis* is rarely found in faecal samples of dogs, and its identification and differentiation from other trichomonads by light microscopy are difficult. Methods of molecular biology are the most effective in this case, because they confirm the presence of the specific species in animal organisms, irrespective of the protozoan form. The aim of this study was to find *P. hominis* in selected dog kennels in North-Eastern Poland. Forty-one faecal samples of dogs from 7 dog kennels were examined. The occurrence of *P. hominis* in 5 faecal samples of dogs with no symptoms of diarrhoea was the first one to be confirmed in Poland.

Key words: *Pentatrichomonas hominis*, real-time PCR, dogs, Poland

Introduction

The *Trichomonadidae* family are protozoans occurring in eukaryotic organisms. *Pentatrichomonas hominis*, which is found in dogs, cats, pigs and cattle, belongs to this family. The *Trichomonadidae* family inhabits the gastrointestinal and urinary tracts and is considered to be an opportunistic commensal which can cause diarrhoea when it inhabits the intestine (Cobo et al. 2003, Gookin et al. 2007, Yun-Ah et al. 2010, Tolbert et al. 2012). The prevalence of *P. hominis* in dogs is not fully known, because it is rarely detected in faecal samples. Trichomonads, including pathogenic and non-pathogenic species, are described in veterinary medicine (Tolbert et al. 2012). In 2003, *Trichomonas foetus* was determined as a direct cause of diarrhoea in cats (Levy et al. 2003). It was stated that *P. hominis* in dogs with diarrhoea occurred more often than *T. foetus* and could cause diarrhoea of unknown aethiology (Gookin et al. 2007, Kim et al. 2010, Tolbert et al. 2012). *P. hominis* was found in children, too, and it was proven that this pathogen was widespread in the environment (Meloni et al. 2011). Identification and differentiation of *P. hominis* from other trichomonads by light microscopy are difficult, because of its close similarity to other protozoans. For accurate identification, methods of molecular biology are the best, as they confirm the presence of the specific species in animal organisms irrespective of the protozoan form. The occurrence of *P. hominis* in dogs in Poland has not been documented. Hence, the aim of this study was to identify this protozoan in selected dog kennels in North-Eastern Poland.

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Table 1. The results of real-time PCR and microscopy for species of parasites in dogs.

No samples	Breed	Sex	Age	Results of real-time PCR for *P. hominis*	Results of microscopy
1.	French Bulldog	female	4 years	–	–
2.		puppy	8 weeks	–	–
3.	German Shepherd	female	4 years	+	–
4.		puppy	9 weeks	+	*Isospora spp.*
5.	Polish Tatra Sheepdog	female	5 years	–	–
6.		puppy	9 weeks	–	*Isospora spp.*
7.		puppy	9 weeks	–	*Isospora spp.*
8.		puppy	9 weeks.	–	*Isospora spp.*
9.	Slovakian Hound	female	3 years	–	–
10.		puppy	8 weeks	–	–
11.		puppy	8 weeks	–	–
12.		puppy	8 weeks	+	–
13.		puppy	8 weeks	+	–
14.		puppy	8 weeks	–	–
15.		puppy	8 weeks	–	–
16.	Bavarian Mountain Hound	female	4 years	–	–
17.		puppy	8 weeks	–	–
18.		puppy	8 weeks	–	–
19.		puppy	8 weeks	+	–
20.		puppy	8 weeks	–	–
21.		puppy	8 weeks	–	–
22.		puppy	8 weeks	–	–
23.		puppy	8 weeks	–	–
24.	English Bulldog	female	5 years	–	–
25.		puppy	4 weeks	–	–
26.		puppy	4 weeks	–	–
27.		puppy	4 weeks	–	–
28.		puppy	4 weeks	–	–
29.		puppy	4 weeks	–	–
30.		puppy	4 weeks	–	–
31.		puppy	4 weeks	–	–
32.	German Shepherd	female	4 years	–	*Toxocara canis, Trichuris vulpis*
33.		puppy	9 weeks	–	–
34.		puppy	9 weeks	–	–
35.		puppy	9 weeks	–	*Toxascaris leonina*
36.		puppy	9 weeks	–	–
37.		young	6 months	–	*Toxascaris leonina*
38.		young	6 months	–	*Toxascaris leonina*
39.		young	6 months	–	
40.		young	6 months		*Toxascaris leonina, Toxocara canis*
41.		young	6 months		*Toxascaris leonina*

Materials and Methods

Forty-one faecal samples collected from 7 dog kennels in North-Eastern Poland were used as the material for the study. The faecal samples were taken from 7 female dogs and their puppies (French Bulldog, German Shepherd, Polish Tatra Sheepdog, Slovakian Hound, Bavarian Mountain Hound). The dogs were held in dog playpens in good environmental conditions. Diarrhoea or symptoms of other diseases were not observed in the animals. They were regularly vaccinated and dewormed with Dehinel, Pratel, Drontal plus (except in the case of the puppies), and the last time the dogs they were dewormed was approximately 6-8 weeks before the samples to be examined were taken. All the puppies were dewormed at the age of 4 weeks. Detailed description of the tested dogs was described in the Table 1.

The real-time PCR was used to identify *P. hominis*. Genomic DNA was extracted from the faecal specimen using a Genomic Mini AX Stool kit (A&A Biotechnology, Gdynia, Poland). The extraction was performed according to the manufacturer's recommendation, and purified DNA was stored for further

studies at a temperature of -20°C. The following *P. hominis*-specific primers were used: Th3 (5'-TGT AAA CGA TGC CGA CAG AG -3') and Th5 (5'-CAA CAC TGA AGC CAA TGC GAG C -3') (Yun et al. 2010). Real-time PCR analysis was performed using FastStart Essentials DNA Green Master (Roche). The reactionary mixture, with a volume of 20 µl, contained approx. 100 ng of the extracted DNA (5 µl), 10 µl of FastStart Essential DNA Green Master; 2x conc., 1 µl of each primer. The volume was adjusted to 20 µl with FastStart Essential DNA Green Master; H_2O, PCR grade. A positive control with the DNA of *P. hominis* was used in each reaction (Instytute Pasteur de Lille, Centre d' Infection et d' Immunitet de Lille (CIIL), Universite Lille Nord de France), and one negative control, in which the DNA was replaced with water, was also used. Reactions were conducted in a Light Cycler Nano System thermocycler (Roche). The applied reaction conditions involved a 1-minute initial denaturation at 95°C and 50 cycles with the subsequent stages of: denaturation at 95°C for 5 min., binding of primers at 64°C for 60 sec. and elongation at 72°C for 2 min. After the last reaction, the chain was finally synthesised at 72°C for 5 min. At the same time microscopic examination was conducted in order to determine the presence of other parasites. Faecal samples collected from dogs were analysed by the flotation method with the use of Darling's solution (50% saturated sodium chloride + 50% glycerol) and examined at a magnification of 400x to search for oocysts and eggs of parasites.

Results

Using the real-time PCR method, it was found that *P. hominis* occurred in 5 samples (12.19%): in the 4-year-old female German Shepherd and her 9-week-old puppy, in two 8-week-old Slovakian Hound puppies and in one 8-week-old Bavarian Mountain Hound puppy (Table 1). Microscopic examination of the specimens did not show *P. hominis*. However, eggs of *Toxocara canis* and *Trichuris vulpis* were found in one (24.39%) out of 7 (100%) of the examined female dogs. The oocysts of *Isospora spp*. were found in the 9-week-old German Shepherd puppy and in three 9-week-old Polish Tatra Sheepdog puppies. Roundworm eggs were detected in four (three had eggs of *Toxascaris leonina* and one of *T. leonina*) young German Shepherds (6-month-old) residing in the same dog farm as the female dog with 9-week-old puppies. However, only one puppy on this farm was infected with *T. leonina* (Table 1).

Discussion

The extraction of *P. hominis* DNA from dog faeces was first carried out in China (Wen-Chao et al. 2014). Puppies at the age of 3 months with chronic diarrhoea were examined. Light microscopy and PCR analysis were used for identification. The effectiveness of these methods in recognizing the protozoan was estimated and it was confirmed that the PCR method was the most accurate. For the first time in Poland, we have shown the occurrence of *P. hominis* but this was indicated only on the basis of the results of examination of 5 faecal samples of dogs from 7 kennels using the real-time PCR method. The sampled dogs showed no symptoms of diarrhoea. Rivera et al. (2008) also stated that identification of *P. hominis* by light microscopy was difficult, because it was impossible to distinguish this species from other trichomonads. Tolbert et al. (2012) found *P. hominis* and *T. foetus* in the examined dogs with diarrhoea. They detected *P. hominis* more often than *T. foetus* in the dogs with diarrhoea and *P. hominis* occurred not only in young dogs at the age of 7 weeks to 6 months, but it occurs in 10-year-old dogs as well. All animals in the examined group experienced symptoms of diarrhoea. Additionally, they stated that the occurrence of a different internal parasite in the dogs with diarrhoea was more common than in the dogs in the control group with no symptoms of diarrhoea. Their results confirm that *P. hominis* in faeces can influence the symptoms of diarrhoea in animals; however, there is still no definite answer to the question. More detailed studies are required, i.e. a greater number of dogs with diarrhoea and without this symptom, in order to confirm the correlation between diarrhoea and the presence of the parasite in dog faeces. Two reports concerning this protozoan found in dog owners with gastrointestinal problems point to the fact that humans can be infected with this protozoan through contact with a dog or cat. Considering the close relation between humans and dogs and the, as yet unknown, prevalence of *P. hominis* in dogs, further studies in this area are advisable (Wen-Chao et al. 2014).

Yun-Ah Kim et al. (2010) examined samples from 14 puppies with symptoms of diarrhoea between the age of 2-9 months and stated that 3 out of 14 puppies (21.4%) were infected with *P. hominis*. Grellet et al. (2013) obtained samples for examination from 215 puppies from 25 dog kennels. The size of the kennel, its location, age of the animals and their body weight were taken into account. The dogs were divided into groups depending on the size of the kennel (the number of the puppies in the litter within a year) and on the weight group of the animals (≤25 kg and ≥25 kg). They showed that 20% (5/25) of the examined

farms were contaminated by trichomonads, and 15.8% of the puppies were infected (34/215). The authors also showed that *P. hominis* occurred in dogs more often than *T. foetus*.

Our study confirmed that P. *hominis* occurred in animals at different ages, but with no symptoms of diarrhoea. The animals were from various kennels. This proves that trichomonads occur in animal organisms but do not cause clear gastrointestinal symptoms.

Keeping pets requires regular control in terms of parasites. The molecular biology methods, i.e. PCR, allowing accurate identification of the protozoan in samples, should be applied to recognize *P. hominis*, as it is more frequently found in dogs and is a diagnostic problem.

Metronidazole is mainly applied to combat *P. hominis*, but it is not included in the majority of anti-parasitic medications for dogs, and therefore an effective method to identify this parasite is required (Yun-Ah et al. 2010).

References

Cobo ER, Campero CM, Mariante RM, Benchimol M (**2003**) Ultrastructual study of a tetratrichomonad species isolated from prepucial smegma of virgin bulls. Vet Parasitol 117: 195-211.

Gookin JL, Stauffer SH, Levy MG (**2007**) Identification of *Pentatrichomonas hominis* in feline fecal samples by polymerase chain reaction assay. Vet Parasitol 145: 11-15.

Grellet A, Polak, B, Feugier A, Boucraut-Baralon C, Grandjean D, Vendewynckel L, Cian A, Meloni D, Viscogliosi E (**2013**) Prevalence, risk factors of infection and molecular characterization of trichomonads in puppies from French breeding kennels. Vet Parasitol 197: 418-426.

Kim YA, Kim HY, Cho SH, Cheun HI, Yu JR, Lee SE (**2010**) PCR detection and molecular characterization of *Pentatrichomonas hominis* from feces of dogs with diarrhea in the Republic of Korea. Korean J Parasitol 48: 9-13.

Levy MG, Gookin JL, Poore M, Birkenheuer AJ, Dykstra MJ, Litaker RW (**2003**) *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the etiologic agent of feline trichomonal diarrhea. J Parsitol 89: 99-104.

Li WC, Gong PT, Ying M, Li JH, Yang J, Li H, Yang ZT, Y, Zhang GC, Zhang XC (**2014**) *Pentatrichomonas hominis*: first isolation from the feces of a dog with diarrhea in China. Parasitol Res 113: 1795-1801.

Meloni D, Mantini C, Goustille J, Desoubeaux G, Maakaroun-Vermesse Z, Chandenier J, Gantois N, Duboucher C, Fiori PL, Dei-Cas E, Doung TH, Viscogliosi E (**2011**) Molecular identification of *Pentatrichomonas hominis* in two patients with gastrointestinal symptoms. J Clin Pathol 64: 933-935.

Rivera WL, Lupisan AJ, Baking JM (**2008**) Ultrastructual study of a tetratrichomonad isolated from pig fecal samples. Parasitol Res 103: 1311-1316.

Tolbert MK, Leutenegger CM, Lobetti R, Birrell J, Gookin JL (**2012**) Species identification of trichomonads and associated coinfections in dogs with diarrhea and suspected trichomonosis. Vet Parasitol 187: 319-322.