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Tritrichomonas foetus in purebred cats in Germany: Prevalence, association with clinical signs, and determinants of infection

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To my parents, with love and gratitude, for believing in me and teaching me to always reach for my dreams.

To my beloved dogs Tris and Lizzy, for faithfully accompanying me throughout the long years of my veterinary education.

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ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
AUTf-1	specific feline *Tritrichomonas foetus* isolate
BVSc	Bachelor of Veterinary Science
C	cytosine
D-1	specific bovine *Tritrichomonas foetus* isolate
Dipl. ACVIM	Diplomate of the American College of Veterinary Internal Medicine
Dipl. ACVN	Diplomate of the American College of Veterinary Nutrition
Dipl. ECVIM-CA	Diplomate of the European College of Veterinary Internal Medicine – Companion Animals
DNA	deoxyribonucleic acid
Dr.	Doctor
Dr. med. vet.	*doctor medicinae veterinariae*
DVM	Doctor of veterinary medicine
et. al.	and others (*et alii)*
FeLV	Feline leukemia virus
FIV	Feline immunodeficiency virus
g	gram
GADPH	glyceraldehyde 3-phosphate dehydrogenase
GPA	grade point average
habil.	*Habilitatus*
ITS1	internal transcribed spacer region 1
ITS2	internal transcribed spacer region 2
kg	kilogram
L.	*Limacus*
µg	Microgram
µl	microlitre
mg	milligram
NFO	Norwegian Forest
P.	*Pentatrichomonas*
PCR	polymerase chain reaction
P	p-value
pH	measure of the acidity of an aqueous solution
PhD	*Philosophiae doctor*
Priv.-Doz.	private lecturer (Privatdozent)
Prof.	Professor
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid

5.8S rRNA	non-coding component of the large eukaryotic ribosomal subunit
18S rRNA	component of the small eukaryotic ribosomal subunit
16S rRNA	component of the small prokaryotic ribosomal subunit
S	Svedberg
S.	*Streptococcus*
T	thymidine
TFITS-F, TFITS-R	specific primers used in PCR assay of *Tritrichomonas foetus*
TFR3, TFR4	specific primer used in PCR assay of *Tritrichomonas foetus*
T. felis	*Trichomonas felis*
T. hominis	*Trichomonas hominis*
T. foetus	*Tritrichomonas foetus*
TM	Trademark
TR7, TR8	specific primers used in the amplification of variable-length DNA repeats from the genome of trichomonads
UK	United Kingdom
USA	United States of America

I. INTRODUCTION

Tritrichomonas foetus (*T. foetus*) is a single-celled protozoan that is well known as the causative agent of the economically devastating venereal trichomonosis in cattle (EMMERSON, 1932; RAE & CREWS, 2006). Recently this parasite has also been identified as an important pathogen in domestic cats (GOOKIN et al., 1999; LEVY et al., 2003). While *T. foetus* infects the bovine urogenital tract, leading to infertility, early embryonic death, and abortion in cows (RIEDMÜLLER, 1928; YULE et al., 1989), in cats, *T. foetus* predominantly causes large-bowel disease and is associated with chronic diarrhoea (GOOKIN et al., 1999; YAEGER & GOOKIN, 2005).

T. foetus was first identified as the cause of feline enteric trichomonosis in the United States of America (USA) in 2003 (LEVY et al., 2003). Since then, the prevalence of this protozoan has been investigated in both pet and purebred cat populations throughout the USA (GOOKIN et al., 2004; STOCKDALE et al., 2009). In Europe, on the other hand, *T. foetus* is a newly emerging enteric pathogen in cats that, to date, has only been investigated in few countries. While *T. foetus* has been reported in diarrhoeic cats in Germany (STEINER et al., 2007; ASISI et al., 2009; SCHREY et al., 2009; KLEIN et al., 2010), no published data exists on the true prevalence of feline trichomonosis in purebred cats and catteries in Germany.

Because *T. foetus* infection in cats is still an emerging disease, the epidemiology of feline trichomonosis is poorly understood and mode of transmission is yet unknown. Observed more frequently in purebred cats (GUNN-MOORE et al., 2007; STOCKDALE et al., 2009), it has not been conclusively established whether this is due to environmental factors or genetic predilection. Furthermore, it still remains unclear whether trichomonosis alone is sufficient to cause clinical signs or whether *T. foetus*-associated diarrhoea is primarily a multifactorial disease process involving concurrent infection with other enteropathogens, host, and environmental factors.

The aim of this thesis, therefore, was (i) to determine the prevalence of *T. foetus* in the faeces of purebred cats in Germany, (ii) to evaluate the association of infection with overt enteric disease and determine whether co-infection with other enteroparasites affects prevalence and severity of clinical signs, and (iii) to identify determinants of infection.

II. LITERATURE REVIEW

1. *Tritrichomonas foetus*

This chapter provides an overview of the evolutionary background, taxonomic classification, morphology, living environment, life cycle, and host range of *T. foetus.*

1.1. Evolutionary background and taxonomic classification

Trichomonads are anaerobic, single-celled, flagellated protozoans. Based on sequencing of rRNA genes, these flagellates have pylogenetically been placed among early-diverging lineages on the eukaryotic evolutionary tree, branching off prior to kinetoplastids which are early mitochondric protozoa (KULDA, 1999; SCHWEBKE & BURGESS, 2004). In the past, the lack of mitochondria observed in trichomonads supported this classification. Instead of mitochondria, trichomonads possess double membrane-bound organelles called hydrogenosomes for energy production (KULDA, 1999). However, due to recent findings in respect to heat-shock protein genes and the biogenesis of hydrogenosomes (BUI et al., 1996; GERMOT et al., 1996; BRADLEY et al., 1997), hydrogenosomes are now thought to share a common ancestor with mitochondria based on similarities in protein import (MULLER, 1997; DYALL & JOHNSON, 2000). Thus, the taxonomy of trichomonads may soon be revised upward (SCHWEBKE & BURGESS, 2004).

Currently, trichomonads are classified in the phylum Sarcomastigophora, order Trichomonadida, family Trichomonadidae (LEVINE et al., 1980). More detailed systematics of trichomonads are largely based on morphological characteristics. Thus, trichomonad species are placed into the three genera *Trichomonas, Pentatrichomonas,* and *Tritrichomonas* according to the number and arrangement of the anterior flagella (LEVINE et al., 1980). A more recent hierarchial classification system ranks trichomonads as [Excavata: Parabasalia: Trichomonadida] (ADL et al., 2005).

In the past, controversy has existed as to the taxonomic differentiation of *T. foetus* and *Tritrichomonas suis* (*T. suis*) which is found in swine (RAE & CREWS, 2006). Based on morphologic and genetic research in 2002, the cattle

pathogen *T. foetus* (Riedmuller, 1928) and *T. suis* (Gruby & Delafond, 1843) were found to be identical using light and electron microscopy as well as three DNA fingerprinting methods and sequence analysis (TACHEZY et al., 2002). Another study in 2005 compared the two species and found no significant differences in morphology, ultrastructure, host specificity, *in vitro* pathogenicity, immunology, and biochemistry (LUN et al., 2005). The authors of both studies conclude that *T. foetus* and *T. suis* belong to the same species (TACHEZY et al., 2002; LUN et al., 2005). TACHEZY and colleagues (2002) further propose that *T. foetus* be adopted as the *nomen protectum* of choice for both porcine and bovine strains and that the name *T. suis* be dropped.

1.2. Morphology

Trichomonads have pleomorphic, spindle-shaped to pyriform cell bodies that measure approximately 10 – 25 micrometres (µm) in length and 3 – 12 µm in width (RIEDMÜLLER, 1928; WENRICH & EMMERSON, 1933). A single nucleus is located at the anterior end of the cell body (WENRICH & EMMERSON, 1933). The Golgi apparatus is very prominent and does not divide during mitosis (BENCHIMOL et al., 2001). The cytoskeleton consists of an axostyle, pelta and costa. The pelta-axostylar complex is a stable structure that supports the cell and participates in karyokinesis (BENCHIMOL, 2004). The rod-shaped axostyle is made up of microtubules and runs along the longitudinal axis of the cell body protruding from the posterior end of the cell. The pelta reinforces the periflagellar canal from which the flagella emerge (WARTON & HONIGBERG, 1979). All genera of trichomonads have a characteristic number of three to five anterior flagella and also often have a posterior recurrent flagellum. The recurrent flagellum constitutes the marginal filament of the undulating membrane (WENRICH & EMMERSON, 1933). Movements of the recurrent flagellum are transmitted to the undulating membrane which then acts as an assistant locomotory organ. The undulating membrane extends along the length of the body and is supported by the costa, a feature characteristic of and unique to trichomonads (BENCHIMOL, 2004). The flagella originate from basal bodies and have axonemes with a 9 + 2 composition of microtubules typically eukaryotic in structure (BENCHIMOL, 2004). The basal bodies are made up of contractile centrin fibers which enable internalization of flagella during pseudocyst formation (STOCKDALE, 2008). Pseudocysts do not have true cyst walls surrounding the

call and form when trophozoites internalise their locomotory organelles in an intact functional form (GRANGER et al., 2000). Trichomonads do not possess mitochondria or peroxisomes (LINDMARK & MÜLLER, 1973), but instead rely on organelles called hydrogenosomes for energy metabolism (BENCHIMOL, 2004).

Organisms belonging to the genus *Tritrichomonas* have three anterior flagella and a recurrent flagellum that extends beyond the undulating membrane (WARTON & HONIGBERG, 1979). The rodlike axostyle is hyaline and extends beyond the length of the cell body (LEVINE, 1985). *T. foetus* has three anterior flagella between 11 – 17 µm. The costa is stout and supports the recurrent flagellum which is about 16 µm long (LUN et al., 2005). The undulating membrane extends about three-quarters of the length of the body and has three to five waves (RAE & CREWS, 2006). The pelta located in the anterior region of the cell body is small (TACHEZY et al., 2002).

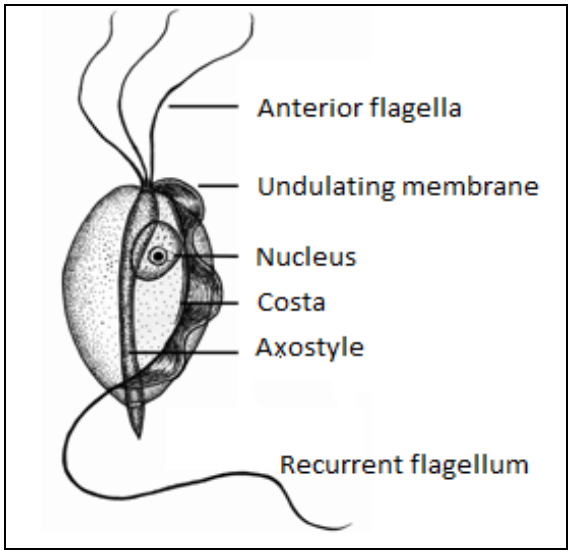

Figure 1: Morphologic features of *Tritrichomonas foetus* (modified from STOCKDALE, 2008)

1.3. Living environment and life cycle

Trichomonads are anaerobic protozoa that are adapted for living in an anaerobic or microaerobic environment such as the vagina (RASHAD et al., 1992) or colon (BORNSIDE et al., 1976). *T. foetus* has a simple life cycle dominated by the trophozoite form. However, under natural and experimental conditions of environmental stress such as a decrease in nutrients, drug

application or abrupt changes in temperature pseudocyst formation can occur (GRANGER et al., 2000; PEREIRA-NEVES et al., 2003). Once considered a degenerate cell form, pseudocysts are currently thought of as environmentally more resistant than trophozoites (PEREIRA-NEVES et al., 2003). Pseudocyst formation has been described in intestinal trichomonads (BOGGILD et al., 2002) and is reversible upon transfer to liquid medium with externalisation of the flagella and resumption of motility (PEREIRA-NEVES et al., 2003).

Trophozoites divide by binary longitudinal fission (WENRICH & EMMERSON, 1933). Replication occurs *via* closed mitosis under participation of the spindle, flagella and axostyle (RIBEIRO et al., 2000). The closed mitosis performed by trichomonads is considered primitive in that the spindle is extranuclear and breakdown of the nuclear envelope does not occur (BENCHIMOL, 2004). *T. foetus* has five chromosomes which become condensed during premitosis. Condensation persists throughout mitosis (RIBEIRO et al., 2002). The nucleolus is duplicated during premitosis and is visible throughout mitosis (BENCHIMOL, 2004). Cell division is preceded by duplication of all skeletal structures, such as the cytoskeleton, the basal bodies, and the flagella in the premitotic phase. The axostyle is very important during mitosis in that it participates in cell shape changes and karyokinesis (BENCHIMOL, 2004). The golgi apparatus does not divide during replication (BENCHIMOL et al., 2001).

Pseudocysts are also able to divide. However, the process differs from the division of trophozoites in that under conditions of environmental stress, *T. foetus* pseudocysts perform nuclear and mastigont division without corresponding cytoplasmic division, thereby creating a polymastigont cell (PEREIRA-NEVES et al., 2003). Return to the trophozoite form upon stable environmental conditions is associated with the budding out of single organisms from the polymastigont cell (PEREIRA-NEVES et al., 2003).

1.4. Host range

T. foetus has been identified in various animals and in humans. The target organ system and pathogenicity of the organism vary according to species (STOCKDALE, 2008).

1.4.1. Cattle

T. foetus is best known for causing economically devastating venereal disease

in cattle. First reported in the late 19th century in France (KUNSTLER, 1888), the pathogenicity of bovine trichomonosis was extensively researched and decribed by RIEDMÜLLER and colleagues (1928) in Germany who proposed the name of *Trichomonas foetus*. *T. foetus* infects the prepucial cavity of bulls and reproductive tract of cows leading to infertility, early embryonic death, abortion, and pyometra in cows. Cows usually clear the infection whereas bulls often become asymptomatic carriers (BONDURANT, 1985). Due to the economic gravity of low fertility rates in infected herds, bovine *T. foetus* infection is a reportable disease in Germany (ANONYMUS; TENTER, 2006). The widespread adoption of artificial insemination over the past decades has resulted in near eradication of bovine trichomonosis in Middle and Western Europe (YULE et al., 1989; TENTER, 2006). In large parts of the world, however, where extensive herd management and natural breeding is still practised, such as Southern Europe, South America and parts of North America, Africa and Australia, *T. foetus* infection in cattle is still prevalent and poses a grave problem due to the economic losses associated with infertility, lack of approved chemotherapy protocols, and the necessity of culling infected animals (BONDURANT et al., 1990; MARTIN-GOMEZ et al., 1998; RAE et al., 2004; TENTER, 2006).

1.4.2. Small ruminants

While small ruminants may be infected with *T. foetus*, the parasite is non-pathogenic in sheep and goats (TENTER, 2006).

1.4.3. Swine

Although the nomenclature still distinguishes tritrichomonads isolated from cattle and swine, bovine *T. foetus* has recently been found to be identical with porcine *T. suis* (TACHEZY et al., 2002; LUN et al., 2005). *T. suis* occurs in the nasal cavity as well as the stomach, small intestine, and caecum of domestic pigs (HIBLER et al., 1960; TACHEZY et al., 2002). Trichomonads were first identified in the porcine stomach in 1843 (GRUBY & DELAFOND, 1843). The organism was later named *Trichomonas suis* by Davaine in 1877 (DAVAINE, 1877). While *T. suis* was once thought to cause atrophic rhinitis in pigs (SWITZER, 1951), further studies were not able to establish a causal relationship, and *T. suis* is now considered a harmless nasal and gastrointestinal commensal in swine (BONDURANT & HONIGBERG, 1994).

1.4.4. Dogs

Intestinal trichomonads have repeatedly been identified in the faeces of young dogs with diarrhoea (SIMIC, 1932; BRUCE, 1941; O'DONNELL, 1954; NIIYAMA et al., 1972; NARAYANA, 1976; TURNWALD et al., 1988; GOOKIN et al., 2005; GRELLET et al., 2010; SCHREY et al., 2010), most of which had coexisting intestinal disease (BRUCE, 1941; O'DONNELL, 1954; TURNWALD et al., 1988). In 2005, the first confirmed case of *T. foetus* was described in a 3-month-old mixed-breed dog with diarrhoea. At the time of diagnosis, the puppy was also coinfected with *Giardia* spp. (GOOKIN et al., 2005). Successful treatment of *Giardia* spp. with fenbendazole did not resolve diarrhoea so that abnormal faecal consistency may have been associated with *T. foetus* infection. Recently, *T. foetus* was detected *via* culture in the faeces of 17.2% of 239 puppies from five of 25 sampled French breeding kennels indicating that *T. foetus* may be a common parasite in dogs. Infected puppies were also significantly more likely to have gastrointestinal problems (GRELLET et al., 2010). Although the culture medium used by GRELLET and collegues (2010) does not support the growth of *Pentatrichomonas* (*P.*) *hominis*, the range of trichomonads morphologically very similar to *T. foetus* for which dogs may act as hosts is unknown. Since morphological species differentiation is unreliable (GOOKIN et al., 2005; GRELLET et al., 2010; SCHREY et al., 2010), studies examining the molecular identity of the enteric trichomonads observed in dogs are required to determine the prevalence and clinical significance of *T. foetus* infection in dogs.

1.4.5. Humans

Only two cases of *T. foetus* infection have been described in humans, both in immune suppressed patients (OKAMOTO et al., 1998; DUBOUCHER et al., 2006). In one case, a man developed *T. foetus*-associated meningoencephalitis following a peripheral stem cell transplantion (OKAMOTO et al., 1998). The other case involved a woman with acquired immune deficiency syndrome (AIDS) who developed *Pneumocytis* pneumonia. *T. foetus* was identified in the bronchoalveolar lavage obtained from this patient (DUBOUCHER et al., 2006). Whether these two cases represent zoonoses or whether a *T. foetus* strain adapted to human host exists is currently not known.

1.4.6. Domestic cats

Approximately a decade ago, *T. foetus* was first identified in the faeces of domestic cats with chronic diarrhoea (GOOKIN et al., 1999; LEVY et al., 2003). Intestinal trichomonads had previously been observed in cats with and without diarrhoea (DA CUNHA & MUNIZ, 1922; SIMIC, 1932; JORDAN, 1956). However, an experimental study and case report 50 years ago failed to establish a causal relationship with intestinal disease (HEGNER & ESKRIDGE, 1935; JORDAN, 1956), and feline trichomonads were considered opportunistic commensals for decades (DIMSKI, 1989; BARR, 1998). Furthermore, early microscopic observation repeatedly described feline trichomonad isolates as having more than three anterior flagella (BRUMPT, 1925; TANABE, 1926; ROMATOWSKI, 1996) which later led to the misidentification of the causative agent of feline trichomonosis as *P. hominis* (ROMATOWSKI, 1996; GOOKIN et al., 1999; ROMATOWSKI, 2000). Recognition of trichomonosis in cats was further complicated by frequent microscopic misinterpretation of trichomonad trophozoites as *Giardia* spp. on direct smears (GOOKIN et al., 1999). However, since genetic identification of *T. foetus* as the cause of feline intestinal trichomonosis in 2003 (LEVY et al., 2003), this protozoan has come to be considered an important worldwide cause of large-bowel disease in domestic cats.

2. Trichomonosis in domestic cats

Since being identified as an emerging gastrointestinal disease in cats at the end of the 20th century, research has focused on both gaining a better understanding on the etiology, epidemiology, and pathogenesis of feline trichomonosis, as well as developing effective means of diagnosis and treatment of infection (GOOKIN et al., 1999; GOOKIN et al., 2002; LEVY et al., 2003; GOOKIN et al., 2006; GOOKIN et al., 2010b; GRAY et al., 2010).

2.1. Etiology and historical review

Although trichomonads were first isolated from the large intestine of domestic cats early in the 20th century (DA CUNHA & MUNIZ, 1922), species differentiation and pathogenicity of feline intestinal trichomonads were only very recently conclusively established (GOOKIN et al., 2001; LEVY et al., 2003). Trichomoniasis was first described in a Brazilian cat by DA CUNHA and MUNIZ

(1922) who named the observed species *Trichomonas felis* (*T. felis*) (DA CUNHA & MUNIZ, 1922). Several years later, BRUMPT (1925) found trichomonads with three to five anterior flagella in both cats and dogs in France and adopted the name *T. felis* for the parasite found in both species. A year later, Tanabe identified pentatrichomonas in a cat which he named *P. felis* assuming that these were the same organisms as reported by Brumpt (TANABE, 1926). While investigating amoebiasis in cats in China, KESSEL (1928) observed several kittens naturally infected with trichomonads characterised by four anterior flagella showing a marked morphological similarity with *Trichomonas hominis* (*T. hominis*). This prompted him to conduct an experimental study on trichomonosis in kittens. Nine naturally infected kittens in this study developed severe diarrhoea accompanied by wasting and death within five to ten days of detection of infection. Of six kittens subsequently inoculated with trichomonads isolated from naturally infected cats, five became infected and also died. Necropsy of all cats demonstrated trichomonads in the inflamed and often necrotic superficial layers of the colon and between cells of the mucosa (KESSEL, 1928). In this study, Kessel also successfully infected and created intestinal disease in kittens *via* inoculation of trichomonads isolated from humans, pigs, monkeys, and white rats. Observing the lack of host specificity, Kessel concluded that (1) trichomonosis was an acquired rather than a primary feline disease, (2) the trichomonad was probably *T. hominis*, and (3) trichomonosis in cats was associated with significant large-bowel disease (KESSEL, 1928). A later experimental study conducted in the USA by HEGNER and ESKERIDGE (1935), however, was not able to establish the pathogenicity of trichomonads in cats. While trichomonads were found in the intestines of previously healthy cats both after being housed with naturally infected cats and after being inoculated with *T. hominis*, none of the felines developed diarrhoea or exhibited any intestinal lesions at necropsy (HEGNER & ESKRIDGE, 1935). In the following years, trichomonads were repeatedly described in both diarrhoeic and non-diarrhoeic cats in the USA (HITCHCOCK, 1953; JORDAN, 1956; VISCO et al., 1978; ROMATOWSKI, 1996) and Europe, including Germany (SIMIC, 1932; WAGNER & HEES, 1935). In 1956, Jordan published a case of intestinal trichomonosis in a 3-year old cat with chronic diarrhoea. However, due to co-infection with *Ancylostoma* spp. and feline panleukopenia virus, diarrhoea could not be attributed to *Trichomonas* spp.. Subsequent transmission experiments using faeces from this cat to infect three kittens were not successful (JORDAN,

1956).

Consequently, most veterinary textbooks published before 2000 questioned the pathogenicity of enteric trichomonads in cats (DIMSKI, 1989; BARR, 1998). In the late 1990s, chronic diarrhoea was repeatedly reported in association with trichomonosis in a total of seven young purebred cats. Based on light microscopic examination of faecal smears, ROMATOWSKI (1996, 2000) identified the trichomonads as *P. hominis*. In 1999, GOOKIN and colleagues (1999) observed trichomonosis in 32 young cats with large-bowel diarrhoea living in multi-cat households. Only few of these otherwise healthy cats had coexisting enteric disease which could have explained the diarrhoea (GOOKIN et al., 1999). With the aim of determining the prevalence of enteric trichomonosis in a healthy, non-diarrhoeic cat population, GOOKIN and colleagues (1999) then attempted to culture trichomonads from a large group of clinically healthy feral and house cats. Trichomonads were not identified in the faeces of any of these cats suggesting that these protozoans are not endogenous intestinal fauna (GOOKIN et al., 1999), as previously assumed (DIMSKI, 1989; BARR, 1998). In contrast to previous descriptions of feline trichomonads, light-microscopic examination of faecal smears of infected cats revealed trophozoites with predominantly three instead of five anterior flagella. This difference in the number of anterior flagella raised questions regarding the true identity of the causative agent of feline trichomonosis (LEVY et al., 2003). In 2001, analysis of the 18S rRNA gene of trichomonad isolates from three naturally infected cats were identical and revealed a 99.9% sequence identity with *T. foetus,* the cause of bovine venereal disease (LEVY et al., 2001). In a subsequent experimental study, axenically cultivated *T. foetus* isolates from a naturally infected kitten reproduced clinically and histologically significant large-bowel disease in four specific-pathogen free cats inoculated with the organism, thus fulfilling Koch's postulates (GOOKIN et al., 2001). Further research revealed that isolated feline trichomonads shared only a low degree of sequence identity of 56.6 – 82.6% with *P. hominis* and that restriction enzyme digest patterns differed significantly (LEVY et al., 2003). In a latter study of cats at risk for or suspected of having clinical trichomonosis, *T. foetus* was identified in 31.0% of 117 and 28.6% of 140 cats *via* PCR, respectively. *P. hominis*, on the other hand, was only observed in the faeces of 1.9% (2/103) and 2.1% (3/140) of investigated cats, respectively, all of which were also positive for *T. foetus*

(GOOKIN et al., 2007a). The combined results of these studies led to the recognition of *T. foetus* and not *P. hominis* as the causative agent of feline enteric trichomonosis.

2.2. Prevalence and geographic distribution

T. foetus was first recognized in cats in the USA (GOOKIN et al., 1999; LEVY et al., 2003). In the past years, reports of feline trichomonosis in many countries indicate that feline *T. foetus* is prevalent among domestic cats worldwide (BRIGUI et al., 2007; GUNN-MOORE et al., 2007; STEINER et al., 2007; BISSETT et al., 2008; BURGENER et al., 2009; VERMEULEN, 2009; KINGSBURY et al., 2010; LIM et al., 2010; XENOULIS et al., 2010b; GALIAN et al., 2011).

2.2.1. North America

To date, two epidemiological studies have investigated the prevalence of *T. foetus* in domestic cat populations in North America (GOOKIN et al., 2004; STOCKDALE et al., 2009). At an international cat show in the USA, 36 of 117 (30.8%) purebred cats tested positive for *T. foetus* via faecal smear, faecal culture, and PCR. Infected cats were identified in 28 of 89 sampled catteries (GOOKIN et al., 2004). A recent survey of 173 pet cats throughout the USA identified *T. foetus* in 9.8% of the pet population using faecal culture confirmed *via* PCR (STOCKDALE et al., 2009). In Canada, *T. foetus* was recently reported in a 14-month-old Abyssinian cat with chronic intermittent diarrhoea (PHAM, 2009).

2.2.2. Europe

Several studies in the past years indicate that feline *T. foetus* is widely distributed throughout Europe. The first case of feline *T. foetus* infection was diagnosed in the United Kingdom (UK) in a Ragdoll kitten with chronic diarrhoea (MARDELL & SPARKES, 2006). In a UK-wide study one year later, Gunn-Moore and colleagues identified *T. foetus* in the faeces of 14.4% of 111 cats with chronic diarrhoea (GUNN-MOORE & TENNANT, 2007). *T. foetus* has also been reported in purebred cats from France. Of 141 cats both with and without diarrhoea from 19 catteries sampled outside of Paris, 15 cats (10.2%) from nine catteries (47.4%) tested positive for *T. foetus* using faecal culture (BRIGUI et al., 2007). A case report of *T. foetus* has also been reported in a cattery in Norway (DAHLGREN et al., 2007). In Italy, enteric trichomonosis was identified in

32.4% of 74 domestic short-hair cats with persistent large-bowel diarrhoea living in a rescue colony in Tuscany (HOLLIDAY et al., 2009). In 2009, 24.4% and 25.7% of 45 and 105 diarrhoeic, mainly purebred cats, respectively, tested positive for *T. foetus* in Switzerland (BURGENER et al., 2009; FREY et al., 2009). An investigation conducted in the Netherlands examined faecal samples from cats with chronic diarrhoea, healthy pet cats, and healthy purebred cats living in catteries around Utrecht (VAN DOORN et al., 2009). The prevalence of *T. foetus* in Dutch mixed-breed and purebred cats established by Van Doorn and colleagues was very low. Using species-specific real-time PCR with confirmation by gel electrophoresis, only one of 53 (1.9%) cats with chronic diarrhoea and none of 54 healthy pet cats tested positive for *T. foetus*. Of the 47 healthy purebred cats sampled in nine catteries, trichomonads were identified in only two cats (4.3%) from two catteries. One of the infected purebred cats was originally from Denmark, the other from Belgium (VAN DOORN et al., 2009). This past year, a study in Greece identified *T. foetus* in 6 (20.0%) of 30 healthy pet cats (XENOULIS et al., 2010b). Intestinal trichomonosis was also recently diagnosed in cats in Spain (ESTEBAN et al., 2010).

Several reports have documented cases of feline trichomonosis in Germany. *T. foetus* was first identified in the faeces of cats from Germany at an international cat show in the USA in 2004 (GOOKIN et al., 2004). In 2007, the organism was found in six (19.4%) of 31 faecal samples examined at a reference laboratory from cats with diarrhoea from Germany and Austria (STEINER et al., 2007). In a study of 103 cats from the general cat population with and without diarrhoea in the Berlin/Brandenburg region, *T. foetus* was cultured from the faeces of three (2.9%) cats (ASISI et al., 2009). In 2009, SCHREY and colleagues (2009) described trichomonosis in three cats with severe diarrhoea. Recently, 9.6% of 376 faecal samples from cats with suspected trichomonosis submitted to a second German reference laboratory tested positive for *T. foetus* (KLEIN et al., 2010). KLEIN and colleagues (2010) also examined the faeces of 297 cats from the general cat population with signs of large-bowel diarrhoea, 3.4% of which tested positive for *T. foetus*.

2.2.3. Asia and Australia

Feline trichomonosis has also been reported in Australia, New Zealand and South Korea. *T. foetus* was first diagnosed in Australia in 16 of 23 cats from an

Ocicat cattery in 2008, two of whom had been purchased from New Zealand and two that were imported from the UK (BISSETT et al., 2008). A later study did not identify trichomonads in any of 134 cattery-housed and shelter cats from four different Australian states (BISSETT et al., 2009). More recently, a case series described *T. foetus* infection in 38 Australian cats (BELL et al., 2010). In New Zealand, *T. foetus* was detected in the diarrhoeic faeces of eight (36.4%) of 22 examined purebred cats from 12 catteries (KINGSBURY et al., 2010). In 2010, Lim and colleagues published the first three cases of feline *T. foetus* infection identified in cats in South Korea (LIM et al., 2010).

2.3. Epidemiology

Although *T. foetus* was only very recently discovered in cats, and much is yet unknown in regard to the origin of infection in cats, disease transmission, and prevalence factors, ongoing studies are leading to a better understanding of the epidemiology of feline trichomonosis (GOOKIN et al., 2004; STOCKDALE, 2008; STOCKDALE et al., 2008; HALE et al., 2009; STOCKDALE et al., 2009; GRAY et al., 2010; SLAPETA et al., 2010).

2.3.1. Origin of feline infection

The origin of *T. foetus* infection in cats is unknown. Several studies have investigated cattle as a possible source of feline infection. An epidemiological study of *T. foetus* in purebred cats found no association of feline trichomonosis with proximity to livestock (GOOKIN et al., 2004). Cross-transmission studies performed by STOCKDALE and colleagues (2007; 2008) indicate demonstrable phenotypic differences in host specificity regarding infectivity and pathogenicity between feline and bovine *T. foetus* isolates. In these two experimental studies, cats were infected with a bovine *T. foetus* isolate and *vice versa* (STOCKDALE et al., 2007; STOCKDALE et al., 2008). Disease manifestation in eight cows inoculated with the cat *T. foetus* isolate AUTf-1 was comparable, but not identical, to the characteristic venereal disease observed upon infection with the bovine *T. foetus* isolate D-1. On histopathology, the epithelium of the uterus differed in both groups of heifers in that all but one cow infected with the cat isolate had an intact uterine surface whereas all heifers infected with the bovine isolate showed a loss of surface epithelium. Furthermore, consistent with reports in the literature, all eight heifers infected with the bovine *T. foetus* isolate cleared

the infection during the 19 weeks post inoculation. In contrast, six of the eight heifers inoculated with the feline *T. foetus* isolate remained culture positive by week 20 (STOCKDALE et al., 2007). Upon inoculation of cats with the bovine *T. foetus* isolate D-1, only two of six cats established an intestinal *T. foetus* infection and tested culture positive at necropsy five weeks *post infectionem*. The cat infected with the feline isolate AUTf-1, on the other hand, tested culture positive on week two and remained culture positive upon weekly faecal sampling. In this cat infected with the feline isolate, *T. foetus* was successfully cultured from the intestinal contents of the ileum, caecum, colon, while the two cats infected with the bovine isolate were culture positive in the caecum only (STOCKDALE et al., 2008). These observations, revealing important biological and pathological differences in disease course, were the first findings indicating that feline and bovine *T. foetus* isolates are host adapted (STOCKDALE et al., 2007; STOCKDALE et al., 2008).

Very recently, molecular characterization of multiple feline *T. foetus* isolates as compared to bovine *T. foetus* isolates using bidirectional sequencing has led to the recognition of significant genetic differences between isolates from cattle and domestic cats. Direct sequencing of the internal transcribed spacer (ITS) region yielded 100% sequence identity of the four cat isolates. Comparison with the sequences of cattle isolates, however, revealed a single nucleotide polymorphism in the internal transcribed spacer 2 (ITS2) region. Subsequent analysis identified this ITS2 thymidine (T) > cytosine (C) polymorphism in all *T. foetus* isolates from cattle and swine available in GenBank (SLAPETA et al., 2010). This same T > C polymorphism in the ITS2 was also observed by Stockdale and colleagues (2009) upon genetic sequencing of 12 isolates from domestic cats. In addition to the ITS2 polymorphism, ŠLAPETA and colleagues (2010) also detected 11 conserved differences between an Australian cat isolate and two bovine isolates at TR7/TR8 variable DNA repeat elements. This is the same marker used by TACHEZY and colleagues (2002) to help prove that *T. foetus* and *T. suis* belong to the same species. Based on the genetic differences observed between feline and bovine isolates, ŠLAPETA and colleagues (2010) have hypothesized that *T. foetus* may be undergoing diversification leading to increased host specificity. Results of studies by STOCKDALE and colleagues (2007; 2008) indicating that direct transmission from cattle to cats is not a likely means of feline *T. foetus* infection

support this hypothesis. However, while STOCKDALE and colleagues (2008) believe that the biological and molecular differences observed between feline and bovine *T. foetus* isolates exceed intra-species variability, ŠLAPETA and colleagues (2010) propose a distinction of species-specific *T. foetus* genotypes, based on the fact that while the isolates appear to be host adapted, host specificity is not yet restricted for either genotype.

2.3.2. Transmission

Transmission of feline *T. foetus* infection between cats is thought to occur faecal-orally (GOOKIN et al., 1999; GOOKIN et al., 2001). Direct contact with fresh, contaminated faeces was long thought to be the sole mode of transmission as *T. foetus*, unlike *Giardia spp*., does not form environmentally stable cysts. However, HALE and colleagues (2009) have since shown that *T. foetus* trophozoites are more resilient outside the host than originally assumed, surviving in moist faeces for seven days at room temperature. A recent Australian study found that *T. foetus* can survive passage through the alimentary tract of two common garden molluscs, the Leopard slug - *Limacus* (*L.*) *maximus* - and the Yellow cellar slug - *L. flavus*. Thus, motile trophozoites were found in 100% (5/5) and 83% (5/6) of *L. maximus* and *L. flavus* slugs fed cat food spiked with 10^6 g^{-1} *T. foetus*. The same study demonstrated that *T. foetus* is viable in wet cat food for five days (VAN DER SAAG et al., 2010). Transmission, thus, may not only be limited to close contact between cats but possibly may also be spread indirectly (HALE et al., 2009; VAN DER SAAG et al., 2010).

Although experimental transmission of *T. foetus* from cattle to cats was possible (STOCKDALE et al., 2008), GOOKIN et al. (2004) found no association between catteries with *T. foetus* and proximity to livestock. Therefore, and because bovine and feline isolates are genetically distinct (see 2.2.1.), direct transmission from cattle to cats is not considered a likely source of feline infection (STOCKDALE et al., 2008).

2.3.3. Prevalence factors

Although, as mentioned above, information on the epidemiology of *T. foetus* infection in cats is still quite limited, a number of predisposing factors for feline trichomonosis are being recognized as more studies are published.

2.3.3.1. Signalment

T. foetus is identified predominantly in young cats. The majority of studies have reported a median age of 12 months or less, with an age range of four weeks to 16 years (GOOKIN et al., 1999; GOOKIN et al., 2004; BURGENER et al., 2009; FREY et al., 2009; HOLLIDAY et al., 2009; BELL et al., 2010; KLEIN et al., 2010; XENOULIS et al., 2010a). Thus, in the UK, 13 of 14 infected cats in a study of 111 faecal samples were one year of age or less (GUNN-MOORE et al., 2007). In a case series of feline trichomonosis in Australia, eight of 13 cats were less than 12 months old at diagnosis (BELL et al., 2010). Of 27 *T. foetus*-positive cats in a Swiss study, 81.5% were in their first year of life (BURGENER et al., 2009). Despite these reports, however, a statistically significant association of *T. foetus*-infection with young age has yet to be established.

The upper age range reported in the studies listed above indicates that *T. foetus* also occurs in older cats. This finding is supported by a study of 74 diarrhoeic cats in a rescue shelter in Italy. Of 24 *T. foetus*-positive cats, 66.7% were over one year of age (HOLLIDAY et al., 2009). Another study of purebred cats living in catteries identified *T. foetus* in 27.3% of adult cats *versus* 17.6% of young cats (GRAY et al., 2010). Of six cats from a healthy general cat population diagnosed with *T. foetus* infection in Greece, five cats were over one year of age, with an age range of six months to nine years. No significant difference in age between infected and non-infected cats was observed (XENOULIS et al., 2010b).

A recent US study that assessed reproductive disease in purebred cats living in catteries at risk for *T. foetus* found male kittens to be more commonly infected than female kittens. Thus, three of 11 male kittens and none of six female kittens tested positive for *T. foetus* (GRAY et al., 2010). A gender predisposition among cats infected with *T. foetus* has not been observed in any other study (GOOKIN et al., 2004; GUNN-MOORE et al., 2007; BURGENER et al., 2009; STOCKDALE et al., 2009).

In most studies of feline *T. foetus* infection, pedigree cats are infected more often than non-pedigree cats (GUNN-MOORE et al., 2007; ASISI et al., 2009; BURGENER et al., 2009; FREY et al., 2009; STOCKDALE et al., 2009; BELL et al., 2010; KLEIN et al., 2010). Of 105 diarrhoeic cats examined for *T. foetus* in Switzerland, all 27 infected cats were purebred (BURGENER et al., 2009). Four of six cats with trichomonosis from Germany and Austria were purebred. One cat

was a domestic short hair; the breed of the sixth cat was not known (STEINER et al., 2007). Of 13 cases of *T. foetus* infection diagnosed in two veterinary hospitals in Australia, 12 were purebred cats (BELL et al., 2010). In a study of 111 faecal samples in the UK, GUNN-MOORE and colleagues (2007) found that pedigreed cats were significantly more likely to be *T. foetus*-positive than domestic crossbred cats. Furthermore, Siamese and Bengal cats were significantly overrepresented among *T. foetus*-positive animals. In the USA, the prevalence of *T. foetus* among 117 purebred cats was 30.8% compared to a prevalence of only 9.8% among 173 cats from the general feline population (GOOKIN et al., 2004; STOCKDALE et al., 2009). Of the 17 *T. foetus*-positive cats in the epidemiologic survey of the US cat population, over 76.5% were purebred cats. Only five were domestic crossbreed cats (STOCKDALE et al., 2009).

On the other hand, two studies have illustrated that *T. foetus* infection is also found in non-pedigreed cats. Thirty-two percent of 74 diarrhoeic domestic crossbreed cats living together in a large outdoor run in a shelter in Italy tested positive for trichomonads (HOLLIDAY et al., 2009). Also, in an early longitudinal study on trichomonosis in cats, 20 of 32 infected cats were domestic shorthairs (GOOKIN et al., 1999).

2.3.3.2. Housing situation

Feline *T. foetus* infection in cats is most often observed in multi-cat households, such as catteries and shelters (GOOKIN et al., 1999; FOSTER et al., 2004). In an epidemiological study of purebred cats in the USA, housing density approached significance as a risk factor for disease (GOOKIN et al., 2004). Of 27 *T. foetus*-infected cats documented by BURGENER and colleagues (2009), 25 lived in multi-cat households. Similarly, 30 of 32 cats with trichomonosis investigated by GOOKIN and colleagues (1999) lived in or were obtained from a cattery or adoption agency prior to diagnosis. Twelve of 13 cats with feline trichomonosis described in an Australian case series lived in large catteries (BELL et al., 2010). While *T. foetus* has seldom been documented in cats in single-cat households (SCHREY et al., 2009), a retrospective evaluation of the living conditions of 104 *T. foetus*-positive cats indicates that the organism is not identified solely in large multi-cat housing situations. In this study, infected cats lived in households with a median of only two cats at the time of diagnosis, with a range of one to fifty cats (XENOULIS et al., 2010a).

Although GOOKIN and colleagues (2004) did not find a significant association of *T. foetus* infection with the management of litter boxes or the type of litter used in catteries, the importance of housing density is thought to be associated with the facilitation of transmission *via* shared litter boxes and is touted as an explanation for the high prevalence of feline trichomonosis among purebred cats which are commonly housed in large groups (GOOKIN et al., 1999; GOOKIN et al., 2004; GUNN-MOORE et al., 2007). The role of housing environment and management *versus* genetic predisposition as key in explaining why pedigreed cats are so strongly overrepresented among *T. foetus*-positive cats is supported by the high prevalence of infection among a large group of non-pedigreed cats living in a high-density environment at a rescue station and sharing a large dirt pit as a latrine (HOLLIDAY et al., 2009). The importance of litter boxes in the epidemiology of feline trichomonosis is indirectly supported by the fact that *T. foetus*-positive cats are uncommonly outdoor cats. Available data indicates that infected cats are most often held indoors necessitating the use of a litter box for defecation (GOOKIN et al., 2004; BURGENER et al., 2009; XENOULIS et al., 2010b). BELL and colleagues (2010) hypothesized that a detection bias for defecation in litter boxes enables recognition of abnormal faecal consistency by owners, thus facilitating diagnosis of trichomonosis in indoor cats. However, *T. foetus* was not identified in the faeces of any of 100 feral cats trapped in the USA as part of a spay-release program, supporting the notion that *T. foetus* is not commonly a pathogen of outdoor cats (GOOKIN et al., 1999).

Only one epidemiological study to date has examined environmental factors related to feline trichomonosis. No association of infection with diet, water source, or other household pets was identified (GOOKIN et al., 2004).

2.4. Pathogenesis

Because feline trichomonosis is still a young disease, the pathogenesis of *T. foetus* infection in cats is not well understood. It is yet unclear whether *T. foetus* alone is sufficient to cause clinical disease, or whether feline trichomonosis is a multifactorial disease process associated with enteric co-infections and host factors (GOOKIN et al., 1999; GOOKIN et al., 2001; BISSETT et al., 2008; STOCKDALE et al., 2009)

2.4.1. Pathologic findings

T. foetus colonizes the ileum, caecum, colon, and rectum (GOOKIN et al., 2001; STOCKDALE et al., 2008). On histopathology, trichomonads most often reside in epithelial secretions in close contact to the mucosal surface and less frequently in the lumen of colonic crypts (GOOKIN et al., 2001; YAEGER & GOOKIN, 2005). *T. foetus* infection is associated with mild to moderate lymphoplasmacytic and neutrophilic infiltration of the lamina propria. Attenuation of the colonic epithelium, crypt epithelial cell hypertrophy, hyperplasia, and increased mitotic activity, loss of goblet cells, and crypt microabscesses are observed in more than 80% of cases. Eosinophilic inflammation may be observed occasionally but is not considered a common feature of *T. foetus* infection (YAEGER & GOOKIN, 2005; SCHREY et al., 2009). In a few cases, *T. foetus* trophozoites also have been observed in the lamina propria. Invasion of the mucosa is associated with more severe histologic lesions such as multifocal mucosal ulcerations and marked transmural inflammation as well as crypt changes (YAEGER & GOOKIN, 2005).

Feline trichomonosis is considered a large-bowel disease with occasional involvement of the ileum. Trichomonads were not observed in the stomach, duodenum, jejunum, or gall bladder of eight *T. foetus*-positive cats at necropsy (GOOKIN et al., 1999). However, exceptions to a sole involvement of the large bowel may exist. Thus, *T. foetus* was recently identified in the duodenum and jejunum of a 15-year old domestic short-haired cat with severe chronic watery diarrhoea associated with weight loss. On histopathology, *T. foetus* infection was associated with moderate to severe eosinophilic inflammation. Enteric co-infections were not identified and clinical small bowel symptoms resolved completely upon administration of ronidazole (SCHREY et al., 2009).

T. foetus has also been identified in the feline reproductive tract. In 2007, DAHLGREN and colleagues (2007) published a case report of a cat with hypersexuality and pyometra living in a *T. foetus*-positive cattery in Norway. Upon ovariohysterectomy, *T. foetus* was identified in the liquid contents of the uterine horns *via* microscopy and PCR. Histological analysis of the uterus was not obtained. Because bacterial culture of the uterine content was positive for *Streptococcus* (*S.*) *canis*, it was not determined whether the pyometra was caused by *S. canis* or *T. foetus*. PCR amplification of faeces from this cat was negative

for *T. foetus* and it was unclear whether uterine trichomonad infection was sexually transmitted or spread from the intestine (DAHLGREN et al., 2007). No other cases of trichomonads involving the reproductive tract in cats have been reported. A recent study of *T. foetus* infection associated with feline reproductive tract disease did not detect any microscopic, immunohistochemical, or molecular evidence of *T. foetus* in the reproductive tract of 40 female and 21 male purebred cats living in 33 catteries. Also, in 22 catteries which housed cats with active or reported intestinal *T. foetus* infection, no effect on breeding success rate or increase in kitten mortality was observed (GRAY et al., 2010).

2.4.2. Parasite-specific mechanisms of pathogenicity

Parasite-specific mechanisms of pathogenicity and host-parasite interactions of feline intestinal *T. foetus* infection have yet to be researched. For venereal *T. foetus* infection in cattle, on the other hand, several experimental infection models exist, and pathogenesis of infection has been extensively studied (FELLEISEN, 1999). Virulence factors recognized in bovine venereal trichomonosis that may also play a role in feline *T. foetus* infection include adhesion to epithelial cells and tissue invasion following enzyme-mediated tissue damage (FELLEISEN, 1999; SLAPETA et al., 2010). In cattle, *T. foetus* is able to adhere to bovine vaginal epithelial cells through filopodia-like protrusions (FELLEISEN, 1999). Similar to trichomonosis in cattle, in cats trichomonads are predominantly found in close proximity to the mucosal surface (FELLEISEN, 1999; YAEGER & GOOKIN, 2005). Thus, *T. foetus* may also be able to attach to epithelial cells of the feline large bowel. In bovines, trichomonad invasion of the placental chorion and of foetal tissue including the intestinal tract has been described (RHYAN et al., 1988; RHYAN et al., 1995). Tissue damage is thought to be mediated by trichomonad proteinases and hydrolases (FELLEISEN, 1999). In two cats, *T. foetus* trophozoites were detected in the deeper intestinal layers of the colon. In one of these cats, severe erosive and ulcerative lesions of the mucosa were observed. The mucosa of the other cat was intact. These histopathological findings which resemble invasive lesions found in aborted bovine foetuses indicate that *T. foetus* may also be capable of direct invasion of the intestinal mucosa in cats (YAEGER & GOOKIN, 2005).

The interaction of *T. foetus* with endogenous bacterial host flora has been discussed as another potential pathogenic mechanism (FOSTER et al., 2004;

PAYNE & ARTZER, 2009). Trichomonads are recognized as obligate parasites that are dependent on obtaining essential nutrients from bacterial flora and host secretions (GOOKIN et al., 1999). Trichomonads may also influence the composition of existing microflora. In human genital infection with the closely related *Trichomonas vaginalis*, trichomonads have a directly deleterious effect on vaginal *Lactobacillus* spp. resulting in a marked rise of pH and an increase in anaerobic bacteria (MCGRORY et al., 1994; PETRIN et al., 1998). Whether the endogenous microflora within the feline large intestine influences the ability of *T. foetus* to establish infection in cats is unknown. However, varied responses to antibiotics in *T. foetus*-positive cats ranging from temporary resolution to exacerbation and prolongation of clinical signs suggest that manipulation of the colonic microflora may directly influence intestinal trichomonads, thereby impacting the course of disease (GOOKIN et al., 1999; FOSTER et al., 2004).

2.4.3. Host-specific determinants of infection

Whether immune status plays a significant role in the pathogenesis of feline *T. foetus* infection is unclear. On one hand, the high prevalence of infection in young cats may indicate an increased susceptibility to trichomonosis due to an immature immune system (GOOKIN et al., 1999). However, an experimental study did not observe exacerbation of disease in cats with trichomonosis that were immunosuppressed *via* administration of prednisolone for 26 days (GOOKIN et al., 2001). Also, no association of *T. foetus* infection with immunosuppressive diseases such as feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) has ever been reported (GOOKIN et al., 1999; ROSADO et al., 2007). Finally, ocular, respiratory, and dermatologic diseases acquired at cat shows which could indicate an impaired immune status were not associated with increased susceptibility for *T. foetus* infection in purebred cats (GOOKIN et al., 2004).

Co-infections with other enteroparasites are frequently observed in cats with *T. foetus* (GOOKIN et al., 2004; STEINER et al., 2007; BISSETT et al., 2008; BURGENER et al., 2009; STOCKDALE et al., 2009; BELL et al., 2010; XENOULIS et al., 2010a). Although it has repeatedly been postulated that infections with other enteropathogens may predispose to trichomonosis (GOOKIN et al., 1999; STOCKDALE et al., 2009), no study has found conclusive evidence to support this claim (GOOKIN et al., 2004).

T. foetus has most often been reported in purebred cats. Although this predisposition is considered to be related to housing conditions and management by most, it has not yet been ruled out that certain breed susceptibilities may exist. In one study, for example, Siamese and Bengal cats were overrepresented (GUNN-MOORE et al., 2007). *T. foetus* has also frequently been reported in Pixie-Bobtails and Russian Blues (ROMATOWSKI, 1996, 2000; BELL et al., 2010).

2.5. Clinical findings

T. foetus has been identified as a cause of chronic or recurrent large bowel diarrhoea in domestic cats (GOOKIN et al., 1999; GOOKIN et al., 2001). Faecal consistency is usually semi-formed to cow-pie like, and less commonly liquid (BURGENER et al., 2009; SCHREY et al., 2009; XENOULIS et al., 2010a). Diarrhoea is often described as characteristically malodorous and may contain fresh blood and mucus. Flatulence and tenesmus are also frequently observed. Severe cases may be accompanied by marked inflammation of the anal region, faecal incontinence and rectal prolaps (GOOKIN et al., 1999; FOSTER et al., 2004; BURGENER et al., 2009; TOLBERT & GOOKIN, 2009; BELL et al., 2010). As feline trichomonosis predominantly affects the large intestine, the majority of infected cats maintain good body condition and appetite without signs of systemic illness (GOOKIN et al., 1999; GOOKIN et al., 2001; TOLBERT & GOOKIN, 2009). Vomiting and weight loss are seldom observed (BURGENER et al., 2009; STOCKDALE et al., 2009; BELL et al., 2010; SCHREY et al., 2010). The severity of clinical signs may be variable, ranging from asymptomatic infection to intractable diarrhoea. Frequently, *T. foetus* infection is characterised by a waxing and waning of diarrhoea (FOSTER et al., 2004; GRAY et al., 2010; XENOULIS et al., 2010b). No abnormalities are routinely noted on haematology and serum biochemistry profile (MANNING, 2010).

2.6. Diagnostic methodology

As trichomonads are not detected on routine faecal analysis, diagnosis of *T. foetus* infection most often requires more specific procedures. A diagnosis can be attained by direct faecal smear (GOOKIN et al., 1999), faecal culture of trichomonads using special culture medium (GOOKIN et al., 2003), or polymerase chain reaction (PCR) amplification of *T. foetus* ribosomal DNA from

faeces using species-specific primers (GOOKIN et al., 2002; GRAHN et al., 2005; FREY et al., 2009). Trichomonads may also be detected on histopathology (GOOKIN et al., 2001; YAEGER & GOOKIN, 2005).

With the exception of endoscopic sampling of the colon, all diagnostic methods rely on the presence of *T. foetus* trophozoites in voided faeces or in faecal material obtained from the intestines *via* rectal swab or faecal loop. Fluctuations in shedding of trophozoites are characteristic of venereal trichomonosis in cattle (SKIRROW et al., 1985). Similarly, in cats shedding of trophozoites in faeces is also thought to intermittently decrease below the detection limit (GOOKIN et al., 2001; STOCKDALE et al., 2008; HALE et al., 2009). Recent antibiotic therapy has been shown to decrease faecal shedding of trichomonads below the detection limit (GOOKIN et al., 1999; FOSTER et al., 2004; TOLBERT & GOOKIN, 2009). A Scottish diagnostic laboratory found that upon repeated sampling of a *T. foetus*-positive cat over a 12-hour period, two of five faecal samples collected tested PCR-negative (VERMEULEN, 2009). In a longitudinal follow-up study, VERMEULEN (2009) collected 12 faecal samples each from two infected cats over the course of four weeks, several of which were negative using real-time PCR from faeces. Similarly, in a study by Gookin et al. (2006) five experimentally infected cats that were repeatedly tested using PCR over a course of 27 weeks tested negative for *T. foetus* on multiple occasions. As each DNA sample was tested for PCR inhibitors prior to testing for *T. foetus*, it is unlikely that these results were all falsely negative (GOOKIN et al., 2006). These findings support the hypothesis that in the course of natural disease faecal shedding of trichomonad trophozoites may vary over the matter of a few hours or days, periodically dropping below the detection limit, similar to the intermittent shedding of trophozoites observed in *Giardia* infections.

2.6.1. Direct faecal smear examination

Identification of motile trophozoites on saline solution-diluted direct faecal smear is the simplest and least expensive method of detecting *T. foetus* in cats. However, this test also has the poorest sensitivity and specificity (HALE et al., 2009). Sensitivity, reported to be as low as 2% in cats with chronic experimentally induced infection (GOOKIN et al., 2001; GOOKIN et al., 2003) and 14% in cats with naturally occurring disease (GOOKIN et al., 2003; GOOKIN et al., 2004), is poor as diagnosis is dependent on the presence of high numbers of viable

trophozoites (HALE et al., 2009). Detection of trichomonads can be optimized by using fresh, unrefrigerated, and moist, preferably diarrhoeic faeces and by analyzing multiple smears (GOOKIN et al., 2001; GOOKIN et al., 2004). Compared to other diagnostic methods, the specificity is very user-dependent as *T. foetus* trophozoites must be distinguished from other protozoal trophozoites, specifically *Giardia* spp. and *P. hominis* (GOOKIN et al., 2003).

2.6.2. Faecal culture

Faecal culture is considered very sensitive and specific for the diagnosis of *T. foetus* in cats (GOOKIN et al., 2003). Long considered the gold standard in the diagnosis of bovine *T. foetus* infection in many countries (GRAHN et al., 2005), culture has also been validated for the diagnosis of *T. foetus* infection in cats (GOOKIN et al., 2003). Two culture mediums are known to support growth of *T. foetus*. While the In Pouch^{TM} feline culture pouch system (Biomed Diagnostics, White City, Oregon, USA) is commercially available and intended for the clinical setting in its ease of use, faecal samples can also be cultured in antibiotic-fortified, modified Diamond's medium which requires sterilization and incubation at 37 °C. Reports regarding comparative sensitivity of the two culture mediums are inconsistent. GOOKIN and colleagues (2004) found no significant difference in rate of detection, whereas HALE and colleagues (2009) determined a significant difference in the cumulative sensitivity of the media. Thus, at a conservative detection limit of 2 x 10^3 organisms per gram (g) of faeces the accumulative sensitivity was found to be 83% and 100% for the InPouch^{TM} and modified Diamond's Medium, respectively, over a six hour period (HALE et al., 2009). In contrast to InPouch^{TM} cultures of reproductive swabs from cattle which have a detection limit of one organsim, GOOKIN and colleagues (2003) determined a detection limit of ≥ 1000 organisms per 0.05 g faeces. Faecal matter hampered detection, and inoculation of more than 0.05 g faeces reduced sensitivity (GOOKIN et al., 2003). Upon inoculation of the InPouch^{TM} culture system with ≤ 0.1 g of faeces, *T. foetus* trophozoites were detected in 20 of 36 positive faecal samples, from which a sensitivity of 56% was calculated (GOOKIN et al., 2003; GOOKIN et al., 2004). Consequently, a single negative test is considered inconclusive. In cattle, testing schemes mandate serial cultures to increase sensitivity of diagnosis. Therefore, in suspected cases of feline trichomonosis, a minimum of three tests over a seven to ten day period has been recommended in

order to rule out *T. foetus* infection (HALE et al., 2009). Specificity of the InPouch^{TM} culture system was found to be high as neither *Giardia* spp. nor *P. hominis* survived in the culture medium for longer than 24 hours. Consequently, a culture yielding motile trophozoites is indicative of *T. foetus* (GOOKIN et al., 2003).

The limitating factor of this diagnostic method is mainly the reliance on the presence of viable trophozoites. As cold temperatures and desiccation are detrimental to the survival of *T. foetus* trophozoites, voided faeces must be moist, fresh, and unrefrigerated. Upon inoculation, faecal cultures must be shipped and stored at room temperature (GOOKIN et al., 2003; HALE et al., 2009).

2.6.3. Polymerase chain reaction

PCR is considered the most sensitive method for detecting *T. foetus* as it does not rely on the presence of viable trophozoites, also detecting dead organisms (GOOKIN et al., 2002; VERMEULEN, 2009). PCR is also considered the method of choice due to it's high specificity.

A variety of PCR assays have been developed for the diagnosis of *T. foetus* both in cattle and cats (HO et al., 1994; FELLEISEN et al., 1998; GOOKIN et al., 2002; BONDURANT et al., 2003; GRAHN et al., 2005). The majority of these tests are based on amplification of sequences of the 5.8S rRNA gene and the flanking internal transcribes spacer regions ITS1 and ITS2 which are highly conserved among various geographically distinct *T. foetus* isolates (FELLEISEN, 1997). Both non-quantitative and quantitative PCR assays exist. These assays are highly sensitive. The absolute detection limit of commonly used primers TFITS-F–TFITS-R and TFR3–TFR4 is as low as one organism per 200 µl (FELLEISEN et al., 1998; GOOKIN et al., 2002). The actual practical detection limit for identification of *T. foetus* in faecal samples, however, is considerably higher. This decrease in the analytic sensitivity of *T. foetus* PCR performed on DNA isolated from faeces is due to the presence of faecal PCR inhibitors not found in other biological substances, such as blood. The composition of faeces is biologically complex, dependent on species-specific intestinal microflora, diet, and concurrent disease. Faecal components such as complex polysaccharides, bile salts, hemoglobin degradation products, phenolic compounds, and heavy metals are often coextracted along with pathogen DNA and may interfere with PCR

performance (GOOKIN et al., 2002; STAUFFER et al., 2008). To minimize false-negative PCR results due to these endogenous PCR inhibitors, optimization of faecal DNA extraction using a specially modified protocol which allows for extendend incubation of extracted DNA with a higher concentration of proteinase K and additional elution is critical (GOOKIN et al., 2002). In addition, various internal and external amplification controls have been developed that enable the detection of faecal PCR inhibitors following extraction of DNA (GRAHN et al., 2005; GOOKIN et al., 2007a; FREY et al., 2009; GRAY et al., 2010). PCR inhibition can be ruled out *via* amplification of either the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (GRAY et al., 2010), bacterial 16S rRNA (GOOKIN et al., 2007b; STAUFFER et al., 2008), or artficial DNA template molecules unrelated to the pathogen (FREY et al., 2009).

Results of a recent Dutch study indicate that PCR of *T. foetus* culture medium previously inoculated with fresh faeces and incubated according to the manufacturer's instructions may be superior to PCR directly from faeces ($p < 0.001$). Thus, in this study, PCR of In Pouch^{TM} medium detected 22 of 24 cats compared to PCR from faeces which detected 12 of 24 *T. foetus*-positive cats and *T. foetus* culture that detected 11 of 24 infected cats. The detection limit of PCR of *T. foetus* culture medium was 10 organisms per 200 µl which was similar to the absolute detection limit of the real-time PCR assay used in the study (VERMEULEN, 2009).

2.6.4. Histopathology

T. foetus trophozoites can also be detected on histopathology of the large intestine. YAEGER and GOOKIN (2005) observed trichomonads in formalin-fixed and paraffin-embedded sections of colon stained with hematoxylin and eosin. Trophozoites were detected in only 55.8% of 43 sections of infected colon, necessitating the examination of six sections to ensure ≥ 95% confidence for the detection of trichomonads (YAEGER & GOOKIN, 2005). Recently, a species-specific fluorescence *in situ* hybridization assay for *T. foetus* was developed enabling the localization and molecular identification of *T. foetus* in formalin-fixed and paraffin-embedded histological specimens (GOOKIN et al., 2010a).

2.7. Treatment

The search for an effective and safe treatment of feline *T. foetus* is ongoing.

T. foetus has exhibited poor *in vitro* and *in vivo* susceptibility to multiple antimicrobial drugs including the two 5-nitroimidazoles metronidazole and tinidazole, drugs commonly used to treat vaginal trichomonosis in humans (GOOKIN et al., 1999; ROMATOWSKI, 2000; GOOKIN et al., 2001; MARDELL & SPARKES, 2006; KATHER et al., 2007; GOOKIN et al., 2007c; STOCKDALE et al., 2009)

Ronidazole, also a 5-nitroimidazole, is currently the drug of choice for the treatment of feline trichomonosis (GOOKIN et al., 2006). The mechanism of action of 5-nitroimidazoles against trichomonads is based on reductive pathways utilized by trichomonads in their energy metabolism (KULDA, 1999). Reduction of 5-nitroimidazole by hydrogenosomal enzymes produces cytotoxic nitro-anion radicals that cause DNA damage and organism death (MORENO et al., 1983; KULDA, 1999; GOOKIN et al., 2010b).

Upon oral application in cats, ronidazole is rapidly and completely absorbed by the proximal small intestine and subsequently metabolised and eliminated through the kidney and liver (ROSADO et al., 2007; LEVINE et al., 2011). A number of reports and studies, none placebo-controlled or double-blind, exist on treatment of feline *T. foetus* with ronidazole (GOOKIN et al., 2006; KATHER et al., 2007; ROSADO et al., 2007; BURGENER et al., 2009; HOLLIDAY et al., 2009; BELL et al., 2010; GOOKIN et al., 2010b; SCHREY et al., 2010; LEVINE et al., 2011). Currently, administration is recommended at a dose of 30 milligrams (mg) per kilogram (kg) once daily over a period of 14 days (LEVINE et al., 2011). Higher dosages of ronidazole have been associated with neurotoxicity in some cats, with neurologic signs occurring a minimum of three days after beginning treatment and resolving one to four weeks after discontinuation (ROSADO et al., 2007). Neurotoxic effects are thought to be dose-dependent and may be attributed to drug accumulation due to the long half-life of ronidazole in cats (LEVINE et al., 2011).

Ronidazole has exhibited good efficacy against *T. foetus* both *in vivo* and *in vitro* (GOOKIN et al., 2006; KATHER et al., 2007). Upon administration of ronidazole, the faecal consistency of infected cats usually shows rapid improvement within days and normalises within the treatment course of two weeks (GOOKIN et al., 2006; BURGENER et al., 2009; HOLLIDAY et al., 2009; BELL et al., 2010). In some *T. foetus*-infected cases, diarrhoea may not resolve

for several weeks following commencement of treatment due to the severity of associated colitis (TOLBERT & GOOKIN, 2009). Symptoms may relapse after treatment with ronidazole as elimination of *T. foetus* is not always successful (BURGENER et al., 2009; GOOKIN et al., 2010b). Infection may resolve after repeating the treatment cycle (GOOKIN et al., 2006).

Treatment failure in cats administered ronidazole for eradication of *T. foetus* infection has been repeatedly documented (GOOKIN et al., 2010b). In a recent retrospective study of 104 cats with trichomonosis, only 59.2% of 49 cats treated with ronidazole had long-term resolution of diarrhoea. Over 32.7% of treated cats showed minimal or no improvement of clinical signs (XENOULIS et al., 2010a). While some of these treatment failures may be attributed to re-infection or inappropriate treatment regime, feline trichomonad infections may also be refractory to ronidazole due to pharmacological resistance of *T. foetus* isolates. Under microaerobic conditions as are present in the colon, trichomonads may be able to develop aerobic resistance to 5-nitroimidazoles by decreasing the activity of their oxygen-scavenging pathway (GOOKIN et al., 2010b). While ronidazole-resistant *T. foetus* isolates have been documented, their prevalence is currently unknown (GOOKIN et al., 2010b).

2.7. Prognosis

In a longitudinal study of cats with feline trichomonosis, 88.5% of 26 cats experienced spontaneous resolution of diarrhoea within two years of onset of clinical signs (FOSTER et al., 2004). Median duration of diarrhoea reported in a recent retrospective study of 104 infected cats was 135 days, with a range of one to 2,880 days (XENOULIS et al., 2010a). Regarding resolution of *T. foetus* infection, FOSTER et al. (2004) found that 54.5% of cats with remission of clinical signs remained asymptomatically infected a median of 39 months after resolution of disease. Therefore, spontaneous elimination of *T. foetus* is considered less likely to occur. Recurrent bouts of diarrhoea following stress or alterations in intestinal flora were common (FOSTER et al., 2004). Finally, it has been postulated that chronic *T. foetus* infection may predispose cats to inflammatory bowel disease (GOOKIN et al., 2001).

III. PUBLICATION

Tritrichomonas foetus in purebred cats in Germany: Prevalence of clinical signs and the role of co-infections with other enteroparasites

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Tritrichomonas foetus infection in purebred cats in Germany: Prevalence of clinical signs and the role of co-infection with other enteroparasites

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The aim of this study was to determine the prevalence of *Tritrichomonas foetus* infection and associated clinical signs in purebred cats in Germany, to investigate the role of co-infection, and identify determinants of infection. Faecal specimens accompanied by epidemiological questionnaires were scored and collected from 230 purebred cats. Faeces were examined for trichomonads and other enteroparasites. The prevalence of *T foetus* was 15.7% among cats and 18.5% among catteries. An abnormal faecal score and history of diarrhoea were observed in 64% and 61% of *T foetus*-positive cats, respectively, and correlated significantly with infection. Co-infection, observed in 36% of *T foetus*-infected cats, was not associated with diarrhoea. Norwegian Forest cats were infected significantly more often than other breeds. No association was found with any environmental factors. This study demonstrated a high prevalence of symptomatic *T foetus* infections in purebred cats in Germany. Co-infection with other enteroparasites did not worsen clinical signs of trichomonosis.

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The single-celled flagellate *Tritrichomonas foetus* has recently been recognised as an enteropathogen in domestic cats.[1–4] *T foetus* predominantly colonises the distal ileum and colon, and infection may manifest as chronic or recurrent large-bowel diarrhoea that is unresponsive to commonly administered antimicrobial drugs.[1,2,5–9]

Enteric trichomonads were long considered naturally-occurring organisms within the feline intestine.[10,11] However, experimental and field studies over the past 10 years have conclusively identified *T foetus* as a non-commensal obligate pathogen in cats,[1–3] and many publications have suggested a strong association between feline *T foetus* infection and chronic diarrhoea.[12–18] Yet, it still remains unclear whether *T foetus* alone is sufficient to cause clinical signs or whether *T foetus*-associated diarrhoea is primarily a multifactorial disease process involving concurrent infection with other enteropathogens, host and environmental factors.[1,2,14,19]

The importance of multi-cat environments not only in catteries but also in shelters in the epidemiology of feline trichomonosis is supported by the high prevalence of *T foetus* (32%) observed among 74 diarrhoeic domestic crossbred cats living in a rescue colony in Italy.[18] However, as the exact transmission mode of feline *T foetus* has yet to be determined and only one epidemiological study to date has examined at-risk housing facilities,[12] other environmental factors cannot be ruled out as potential influences on prevalence and should be further investigated.

Studies in Europe investigating feline *T foetus* infection have focused primarily on cats with chronic diarrhoea, and the pathogen has been detected in the faeces of 2–32% of cats in the UK, Italy, Switzerland and the Netherlands.[13,16–18,20,21] *T foetus* was first found in the faeces of several pedigreed cats from Germany attending an international cat show in the USA in 2001.[12]

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More recently, the protozoan was found in the faeces of 6/31 (19%) cats with chronic diarrhoea in Germany and Austria.[22] To date, however, no published data exists on the prevalence of *T foetus* infection in cat populations in Germany.

The purpose of this study was (1) to determine the prevalence of *T foetus* in the faeces of purebred cats in Germany, (2) to evaluate the association of infection with overt enteric disease and determine whether concurrent infection with other intestinal parasites exacerbates clinical signs of feline trichomonosis, and (3) to identify factors associated with *T foetus* infection.

Materials and methods

Data collection

Freshly voided faecal specimens and epidemiological questionnaires were collected from 230 purebred cats representing 124 catteries at five regional cat shows throughout Germany between April and August 2008. Faeces were collected from cats that were present at the cat show as well as other cats of the participating catteries not attending the cat show. Cats with a completed survey and an unrefrigerated fresh faecal specimen less than 4 h old were included in the study.

Epidemiological survey
The cats' owners were asked to fill out an epidemiological questionnaire providing detailed information on signalment, diet, water source, housing situation, direct and indirect contact with livestock and other pets, as well as medical history (Appendix I). Specifically, survey questions were designed to determine the onset, severity, and frequency of gastrointestinal signs and record any medications administered within the preceding 6 months.

Faecal scoring
Faecal consistency was evaluated at the time of collection by the primary investigator (KK) and scored based on a modified continuous faecal scoring system for dogs and cats (Purina Fecal Scoring System for Dogs and Cats, Nestle-Purina) with a score of 1, representing liquid diarrhoea; 2, pudding consistency diarrhoea; 3, loose but formed faeces; and 4, firm faeces. Faecal specimens with a score of 1 and 2 were considered diarrhoeic, faecal specimens with a score of 3 and 4 were considered non-diarrhoeic.

T foetus *faecal culture*
An aliquot of fresh faeces ($\leq$0.5 g) from each cat was inoculated into InPouch TF culture medium (Biomed Diagnostics). Pouches were stored vertically in the dark at room temperature and examined for motile trophozoites using light microscopy starting 48 h post inoculation. Microscopic evaluation was performed every day from day 2 through day 6 and every other day from day 6 through day 12. Each pouch was examined under low power (100× magnification) for $\geq$ 5 min, concentrating on the bottom portion and edges of the pouch. Upon identification of trophozoites, 400× magnification was used for confirmation of trichomonads. Observation of $\geq$ 1 motile trichomonad was considered a positive result.[23]

Immediately following processing for faecal culture, the remaining faeces were placed in a cooler and refrigerated at 7°C within 6 h of collection.

Giardia *and* Cryptosporidium *immunoassays*
Fresh, refrigerated faecal specimens were examined for the presence of *Giardia* and *Cryptosporidium* species antigens by monoclonal microplate immunoassays (ProSpecT Giardia Microplate Assay, ProSpecT Cryptosporidium Microplate Assay, Remel) within 36 h. All tests were performed and visually interpreted according to manufacturers' instructions.

Faecal flotation
A portion of refrigerated faeces was examined within 72 h of collection for the presence of nematode eggs, *Giardia* species cysts and coccidian oocysts using zinc sulfate centrifugation flotation as previously described.[24]

T foetus *polymerase chain reaction (PCR) and trichomonad typing and sequencing*
Faecal specimens were frozen at −70°C for up to 1 month prior to DNA extraction. Faecal DNA was isolated from all 230 specimens using the QIAamp DNA Stool Mini kit (Qiagen) according to manufacturer's instructions with the following modifications: 20 µl of proteinase K was incubated with the extracted solution at 56°C for 1 h prior to adding the lysis solution AL, and two washes were performed with buffer AW1.

PCR amplification followed the conditions established by Grahn et al with minor modifications.[25] Three stool sample DNA template volumes were used (0.5 µl, 2.0 µl, and 8.0 µl), and water was decreased accordingly to maintain sample volume and appropriate reaction concentrations. Bovine serum albumin (BSA) was added to a final reaction concentration of 0.1% and thermal profile cycling was increased from 30 to 35. Products were size separated on an ABI 3730 DNA analyzer with GeneScan LIZ 500 size standard (Applied Biosystems). Exact sizes were determined with STRand analysis software.[26]

To verify the identity of amplified trichomonads, representative PCR amplicons were directly sequenced. Products were amplified using unlabelled primers under reaction conditions listed above to maximise sequence read length and obtain double stranded sequence. Twenty microlitres of PCR product was prepared for sequencing using ExoSAP-IT (USB) to remove unincorporated primers and dNTPs. Sequencing was performed using the Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing products were cleaned over Centri-Sep Spin Columns (Princeton Separations) and separated on an ABI 3730 DNA Analyzer. Sequences were visualised using the Sequencher Software (Gene Codes Corp), and sequence identity was confirmed with a BLAST search.[27]

Table 1. Association of feline *T foetus* infection with diarrhoea based on faecal score and a history of diarrhoea in the past 6 months.

		T foetus positive		*T foetus* negative	
		Cats ($n = 36/230$)	Catteries ($n = 23/124$)	Cats ($n = 194/230$)	Catteries ($n = 101/124$)
Faecal score*	1	1 (3%)		6 (3%)	
	2	22 (61%)		45 (23%)	
	3	9 (25%)		56 (29%)	
	4	4 (11%)		87 (45%)	
History of diarrhoea in past 6 months	Yes	22 (61%)	16 (70%)	73 (38%)	45 (45%)
	No	14 (39%)	7 (30%)	121 (62%)	56 (55%)

*Faecal score based on a modified continuous faecal scoring system: 1 = liquid diarrhoea, 2 = pudding consistency diarrhoea, 3 = loose but formed faeces, 4 = firm faeces.

Statistical analysis

Statistical analyses were conducted using commercial software (SPSS Version 17, SPSS; StatCalc 5.4.1, AcaStat Software; Stata Version 9.1, Stata Corp). Descriptive statistics were performed for all variables. Prevalence was determined with a 95% confidence interval (CI). Continuous data was examined for normality by the Kolmogorov–Smirnov test. For variables non-Gaussian in distribution, the Mann–Whitney test was used to compare between groups. Categorical data was analysed using a χ^2 test. In 2×2 contingency tables with any expected cell values < 5, Fisher exact two-tailed results were used. Prevalence odds ratios (PORs) and 95% CI were calculated where appropriate. All variables with a P-value of < 0.2 were further analysed using a multivariate logistic regression model with robust variance estimation to account for the cluster effect of catteries. A P-value of < 0.05 was considered statistically significant.

Results

Study population

Freshly voided faecal specimens and a completed epidemiological survey were collected from 230 purebred cats representing 124 catteries in Germany. The number of cats sampled per cattery ranged from one to 11 (median 1, mean 1.9). Surveyed catteries contained a median of nine cats with a range of one to 33 cats per household. Ninety of the cats were male (63 intact, 27 castrated) and 136 were female (108 intact, 27 spayed). The gender of four cats was not recorded. The age of sampled cats ranged from 3 weeks to 18 years (median of 1.1 years, mean of 2.3 years). The age of two cats was unknown. The study population was made up of 25 different breeds. Maine Coon cats ($n = 77$), British Shorthairs ($n = 40$), Birmans ($n = 18$), and Norwegian Forest (NFO) cats ($n = 15$) were over represented.

Prevalence of T foetus *in Germany*

The overall prevalence of *T foetus* infection was 15.7% (36/230 [95% CI 11.5–20.9]) among individual cats and 18.5% (23/124 [95% CI 12.7–26.3]) among catteries. Diagnosis of *T foetus* was based on either positive faecal culture results (29/230) or demonstration of *T foetus* DNA via PCR of faecal DNA (28/230). No significant difference was observed in the geographical distribution of *T foetus* infection throughout Germany.

Clinical signs

The prevalence of diarrhoea in this study was based on faecal score and data obtained from the epidemiological survey (Table 1). An abnormal faecal consistency (corresponding to a faecal score of 1 and 2) on the day of sampling at the cat show was observed in 64% (23/36) of *T foetus*-positive cats and correlated strongly with the detection of *T foetus* ($P < 0.001$; POR 3.98; 95% CI 1.90–8.33). Firm faeces (corresponding to a faecal score of 4) were noted in only 11% (4/36) of infected cats. A history of diarrhoea in the past 6 months, documented in 61% (22/36) of *T foetus*-positive cats, was also significantly associated with *T foetus* infection ($P = 0.027$; POR 3.15; 95% CI 1.14–8.74). Likewise, 70% (16/23) of *T foetus*-positive catteries reported a history of diarrhoea among cats within the cattery in the past 6 months ($P = 0.010$; POR 3.22; 95% CI 1.23–9.87). *T foetus*-infected cats without a history of diarrhoea were significantly more likely to have diarrhoea on the day of the cat show than *T foetus*-negative cats without a history of diarrhoea ($P = 0.002$). No correlation was observed between the age of *T foetus*-positive cats and either faecal score or a history of diarrhoea. Frequency of defecation, faecal incontinence, faeces containing mucus and blood, or weight loss were not associated with *T foetus* infection.

Other enteric parasites

Enteric parasites other than *T foetus* were identified in 49/230 faecal specimens (21.3%). Of the 36 cats that tested positive for *T foetus*, 13 (36.1%) were co-infected with other parasites. No other enteric parasite other than *T foetus* was associated with an abnormal faecal score and a history of diarrhoea. Co-infection with

Giardia species and *T foetus* was observed in 10/230 cats from 6/124 catteries, representing 27.7% (10/36) of *T foetus*-positive cats. Six cats from four catteries were positive for *T foetus* and *Isospora* species, representing 16.7% (6/36) of *T foetus*-positive cats. Of these cats, three were co-infected with *T foetus*, *Giardia* species and *Isospora* species. Faecal consistency did not differ in *T foetus*-positive cats co-infected with *Giardia* species and/or *Isospora* species as compared to cats infected solely with *T foetus*. The small number of faecal specimens positive for both *T foetus* and *Cryptosporidium* species ($n = 1$) precluded determination of any association between co-infection with this parasite and faecal consistency.

Factors associated with T foetus *infection prevalence*

No association was found between gender and *T foetus* infection. The age of *T foetus*-positive cats ranged from 3 weeks to 7.2 years (median age: 1.0 year). Nearly 70% (25/36) of *T foetus*-positive cats were ≤1 year old ($P = 0.034$; POR 0.85; 95% CI 0.73–0.99), and the prevalence of *T foetus* infection decreased with age (POR = 0.83; 95% CI 0.70–0.98). *T foetus* infection was identified in cats of 11/25 breeds (Table 2). Approximately 67% (10/15) of NFO cats and 88% (7/8) of NFO catteries throughout Germany tested positive for *T foetus*. NFO cats had a significantly higher prevalence of *T foetus* infection than other breeds ($P < 0.001$, POR 25.89; 95% CI 7.63–87.72). No significant association was observed between *T foetus* infection and any other breed.

T foetus infection was not correlated with any environmental factor, including diet, water source, direct and indirect contact to livestock or other pets, proximity to agricultural facilities, ratio of cats to litter boxes, and housing density as determined by number of cats per household and square metres per cat.

Discussion

In this study, 15.7% (36/230) of examined purebred cats and 18.5% (23/124) of surveyed catteries in Germany tested positive for *T foetus*. While *T foetus* has previously been identified in German cats, no published studies exist on the prevalence of *T foetus* in Germany. Furthermore, only two other epidemiological studies examining the overall prevalence of *T foetus* in a large population of both diarrhoeic and non-diarrhoeic cats, both conducted in the USA, have been published to date.[12,14] *T foetus* was identified in 31% of 117 purebred cats from 89 catteries at

Table 2. Breed distribution of *T foetus*-positive cats and catteries (values are expressed as a fraction and percentage [%] of the total number of cats and catteries of each breed).

Breeds ($n = 25$)	*T foetus* positive					
	Cats ($n = 36$)			Catteries ($n = 23$)		
Maine Coon	5/77	(6.5%)	$P = 0.018$*	4/45	(8.9%)	
British Shorthair	7/41	(17.1%)		2/18	(11.1%)	
Birman	0/18	(0.0%)		0/10	(0.0%)	
NFO cat	10/15	(66.7%)	$P < 0.001$*	7/8	(87.5%)	$P < 0.001$*
Burmese	4/9	(44.4%)		2/4	(50.0%)	
Balinese	2/8	(25.0%)		1/2	(50.0%)	
Persian	1/8	(12.5%)		1/8	(12.5%)	
Bengal	2/7	(28.6%)		1/3	(33.3%)	
Thai	1/7	(14.3%)		1/2	(50.0%)	
Somali	2/6	(33.3%)		2/3	(66.6%)	
Siberian cat	1/6	(16.7%)		1/3	(33.3%)	
Devon Rex	0/5	(0.0%)		0/2	(0.0%)	
Oriental Shorthair	1/3	(33.3%)		1/2	(50.0%)	
Abyssinian	0/3	(0.0%)		0/2	(0.0%)	
Highlander	0/3	(0.0%)		0/2	(0.0%)	
Siamese	0/3	(0.0%)		0/1	(0.0%)	
Savannah	0/2	(0.0%)		0/1	(0.0%)	
Tonkinese	0/2	(0.0%)		0/2	(0.0%)	
Neva Masquerade	0/1	(0.0%)		0/1	(0.0%)	
Sphinx	0/1	(0.0%)		0/1	(0.0%)	
Russian Blue	0/1	(0.0%)		0/1	(0.0%)	
Oriental Longhair	0/1	(0.0%)		0/1	(0.0%)	
Selkirk Rex	0/1	(0.0%)		0/1	(0.0%)	
Mandarin Oriental	0/1	(0.0%)		0/1	(0.0%)	
Ragdoll	0/1	(0.0%)		0/1	(0.0%)	

*P-values < 0.05 are shown.

an international cat show in the USA.[12] Although the current study investigated a similar cat population using similar methodologies, the prevalence of *T foetus* observed in purebred cats in Germany was much lower. Inherent variations between the two populations might explain some of the differences in the results between the two studies. However, it is also possible that the prevalence of *T foetus* may have been underestimated in our study. Because the present study relied on testing of a single faecal specimen and did not exclude cats that received antibiotics within 2 weeks of faecal testing, the prevalence of *T foetus* in purebred cats in Germany may actually be higher than our results indicate.

In a recent investigation of 61 purebred cats in 36 catteries in the USA, a history of diarrhoea was reported by owners in only 25% of the study population and was not significantly associated with trichomonosis.[28] While it has been postulated that chronic asymptomatic *T foetus* infections may be quite common,[6] the current study identified very few animals with subclinical trichomonosis. *T foetus* infection correlated strongly with an abnormal faecal score, and of the 36 cats that tested positive for *T foetus*, only four cats (11%) had firm faeces as determined by faecal score on the day of the survey. A larger percentage of *T foetus*-positive cats (14/36, 40%), on the other hand, had no history of diarrhoea in the past 6 months. This suggests that in asymptomatic cats, bouts of diarrhoea may readily be triggered by environmental stress, such as a cat show, explaining the waxing and waning of clinical signs commonly seen in feline *T foetus* infections.

Although enteric co-infections have frequently been documented in *T foetus*-infected cats,[1,2,12,14,16] their association with clinical signs has not been well established. A study of experimental *T foetus* infection observed more severe diarrhoea and increased shedding of trichomonads in four cats concurrently infected with *Cryptosporidium* species.[1] In contrast, the current study of naturally infected cats found no association between co-infection with other enteric parasites and *T foetus*. Furthermore, *T foetus*-associated diarrhoea was not exacerbated by co-infection with *Giardia* species or coccidia, and *T foetus* infection alone was sufficient to cause significant clinical signs.

Several studies have suggested that *T foetus* infection is primarily a disease of young cats and kittens.[2,13,14,16] In agreement with current views, the present study was able to show a significant association of *T foetus* infection with young age. Nearly 70% of infected cats were ≤ 1 year, and the prevalence of disease decreased with advancing age. Of the 28 cats older than 5 years sampled in this study, only one cat tested positive for *T foetus*. In contrast, a recent investigation in a rescue colony in Italy found that 67% of 24 infected cats were over 1 year of age.[18] While these results clearly indicate that older cats are also at risk for disease, younger cats may be more vulnerable to disease due to an immature immune system.[2] Alternatively, the high prevalence of *T foetus* infection among kittens and young cats may reflect the time point at which transmission of *T foetus* within a cattery is most likely to occur. It is plausible that the risk of faecal–oral transfer of *T foetus* is greatest between an infected queen and her kittens and, subsequently, among kittens within that litter. Interestingly, severity of *T foetus*-associated diarrhoea was not age-related, and older infected cats in this study were just as likely to have an abnormal faecal score or history of diarrhoea as young animals.

NFO cats and catteries in Germany were at a significantly higher risk of *T foetus* infection than other breeds. Thus, *T foetus* was identified in nearly 67% (10/15) of NFO cats and 88% (7/8) of NFO catteries. Infected catteries were distributed throughout Germany and no association was found between NFO cats regarding recent ancestry or breeding programmes. Because of the high prevalence of *T foetus* among purebred cats, the possibility that some breeds may have a genetic predisposition for *T foetus* infection has been repeatedly discussed.[13,14] Although one study in the UK observed a high rate of *T foetus* infection among Siamese and Bengal cats,[13] this finding was not documented in other studies of purebred cats including the current investigation.[14,16,20] Two recent studies of diarrhoeic cats in Switzerland identified *T foetus* in NFO cats, but did not indicate that this breed was over represented.[16] Therefore, while a genetic predisposition of NFO cats in Germany cannot be ruled out at this time, other possible associations among the infected NFO cats in our study such as country of acquisition or management within these NFO catteries should be more closely investigated.

Indoor high density multi-cat housing, as commonly found in catteries and shelters, is regarded as a key risk factor for feline trichomonosis. Although the exact mode of transmission of *T foetus* is yet unknown, crowding may increase infection pressure by leading to an increased risk of oral–faecal contact via shared litter boxes.[12,29] While the square feet of facility per cat approached significance ($P = 0.056$) in another study of *T foetus* in purebred cats living in catteries,[12] the current study was not able to identify an association between *T foetus* and housing density. Purebred catteries surveyed by Gookin et al (2004) contained a median number of 16 cats with a range of one to 59 cats per household and a median of 71.4 square feet of facility per cat in infected catteries.[12] In contrast, the housing density in German catteries documented in this study was much lower. Sampled catteries that tested positive for *T foetus* contained a median number of seven cats with a range of one to 33 cats per household and a median of 129 square feet of facility per cat. It is plausible that the housing density observed in this study was not high enough to influence disease prevalence. It is also possible that the questionnaire requesting information on the number of cats per household and square metres per facility was not answered honestly for fear of repercussions related to animal rights violations.

In addition, no correlation was observed between *T foetus* infection and the ratio of cats to litter boxes. Similarly, Gookin et al (2004) was not able to identify an association with litter box management or the type of litter used.[12] These results are unexpected considering the

presumed environmental fragility of *T foetus* trophozoites. Because trichomonads do not form environmentally stable cysts, only freshly voided faeces are thought to contain infectious organisms,[12] and shared litter boxes have been implicated in the faecal–oral spread of *T foetus*.[29] Recently, however, an experimental study showed that *T foetus* is more resilient in cat faeces at room temperature than previously assumed. In moist faeces, the organism can survive for at least 7 days,[30] indicating that the faecal–oral spread of the organism may not require contact with a fresh faecal specimen. Thus, it is conceivable that transmission of *T foetus* is not limited to immediate oral contact with faeces in the litter box. Environmental contamination with faeces, especially diarrhoea, from infected cats may contribute to the spread of *T foetus* and may require more stringent sanitation measures than previously assumed.

In conclusion, this study found a high prevalence of *T foetus* infection in purebred cats throughout Germany. Clinical disease was very common, indicating that *T foetus* infection should be considered an important differential diagnosis for chronic diarrhoea in purebred cats in Germany, especially in young animals. Findings suggested that in asymptomatic cats, bouts of diarrhoea may potentially be brought on by stress. Co-infection with *Giardia* species or coccidia was not associated with the prevalence or severity of diarrhoea in *T foetus*-positive cats, indicating that *T foetus* infection alone is sufficient to cause clinical disease. No evidence was found to support the notion that clinical signs of *T foetus* infection are exacerbated by co-infections with other enteric parasites.

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Appendix 1. Copy of survey used to obtain epidemiological information from participating cats and catteries

Date:_____

Owner Information

Name _____

Phone number _____

Postal code _____

Cattery number **(this will be filled out by the investigator)** _____

Information of Cat

Cat's name _____

Date of Birth or Approximate Age (years) _____

Breed _____ Sex: _____ Intact or Neutered: _____

Cat's faecal score **(this will be filled out by the investigator)** 1 2 3 4

Living Environment:

What do you feed your cat? commercial diet □ homecooked diet □ raw diet □

Water sources for your cat at home: tap water □ toilets □ ponds or pool □ bottled water □ bird bath □ other □, please specify: _____

My cat is housed only indoors □ free outdoor access □ indoors with restricted outdoor access (e.g. outdoor enclosure) □

Total number of cats currently living in household? _____

Any other pets in household besides cats? Yes □ No □

If yes, what types and numbers of pets? _____

Total number of litter boxes in household? _____

How many litter boxes does your cat have permanent access to? _____

How many other cats does your cats share these litter boxes with? _____

Does your cat have access to other cats outside your cattery? Yes □ No □

If yes, what type of contact (e.g. breeding purposes, contact to other outdoor cats)? _____

How close (in kilometers) is your home to livestock (cows/sheep/goats)? _____

Has your cat had any direct physical contact with livestock? Yes □ No □

Approximate size of your cat's permanent living space (in square meters)? _____

How many other cats does your cat share this living space with? _____

Medical History:

Has your cat had diarrhoea within the last six months? Yes □ No □

If yes, …

Duration of diarrhoea (in weeks)? _____

Does your cat currently have diarrhoea? Yes □ No□

How many times a day did/does your cat have diarrhoea? 1x □ 2x □ 3x□ 4x □ ≥ 5x□

Any loss of weight in this cat within the past 6 months? Yes □ No □

Any blood (bright red) in this cat's stool within the past 6 months? Yes □ No □

Any mucus (slimy, gelatinous material) in this cat's diarrhea within the last six months? Yes □ No □

Any dripping of faeces from anus in this cat within the last six months? Yes □ No □

Was diarrhoea medically treated? Yes □ No □

If yes, which medication? _____ Did diarrhoea resolve upon medical therapy? Yes □ No □

Was your cat examined for gastrointestinal parasites within the past six months? Yes □ No □

If yes, diagnosis? *Giardia* □ *Cryptosporidium* □ Coccidia □ *Tritrichomonas foetus* □ no parasites found □

Has this cat ever been diagnosed with *Tritrichomonas foetus?* Yes □ No □

Any administration of antibiotics to this cat within the past 6 months? Yes □ No □

If yes, which antibiotic? _____ time point and duration of treatment? _____

Any administration of antiparasitic medications (e.g. dewormer) to this cat within the past 6 months?

Yes □ No □

If yes, …

Which medication? _____

Reason for administration of medication? Routine □ Other □, please specify _____

Duration of treatment? _____

Have any other cats in household had diarrhoea within the past six months? Yes □ No □

If yes, number of other cats that have had diarrhoea _____

Were other cats in your household examined for gastrointestinal parasites within the past six months?

Yes □ No □

If yes, diagnosis? *Giardia* □ *Cryptosporidium* □ Coccidia □ *Tritrichomonas foetus* □ no parasites found □

Has any cat in your cattery ever been diagnosed with *Tritrichomonas foetus?* Yes □ No □

If yes, how many? _____

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IV. DISCUSSSION

1. Prevalence in Germany

This was the first study to examine the prevalence of *T. foetus* in the general purebred cat population in Germany. Of 230 examined purebred cats from 124 surveyed catteries, 15.7% (36/230) tested positive for *T. foetus*. In past years, *T. foetus* has repeatedly been identified in cats in Germany (GOOKIN et al., 2004; STEINER et al., 2007; ASISI et al., 2009; SCHREY et al., 2009), and German reference laboratories are now offering commercial *T. foetus*-specific faecal PCR assays. Prevalence of *T. foetus* in diarrhoeic faecal samples predominantly from German cats submitted to one such laboratory for faecal PCR was 9.0% (166/1840) (GALIAN et al., 2011). This prevalence is lower than reported in the current study and compares to the prevalence of 9.8% among 173 pet cats in a recent epidemiologic study which examined cats in veterinary clinics throughout the USA (STOCKDALE et al., 2009). While the current study drew samples solely from the purebred cat population, only 31 of the 173 cats surveyed by STOCKDALE et al. (2009) were purebred or a purebred cross. Yet, of the cats testing positive for *T. foetus,* 70.6% (12/17) were purebred (STOCKDALE et al., 2009). Similarly, in another recent study on feline *T. foetus* conducted by a German reference laboratory, prevalence of *T. foetus* upon examination of all submitted faecal samples was 9.6% (36/376). Upon examination of faecal samples solely from purebred cats, prevalence of *T. foetus* was as high as 17.3% (KLEIN et al., 2010). In agreement with this finding, most past studies have identified trichomonosis in pedigreed cats more frequently than in non-pedigreed cats (GUNN-MOORE et al., 2007; BURGENER et al., 2009; FREY et al., 2009; VAN DOORN et al., 2009).

Only two other epidemiologic studies have examined the overall prevalence of *T. foetus* in a large population of both diarrhoeic and non-diarrhoeic cats (GOOKIN et al., 2004; STOCKDALE et al., 2009). At an international cat show in the USA, *T. foetus* was identified in 30.8% of 117 purebred cats from 89 catteries by direct smear, faecal culture, and PCR assay from faeces (GOOKIN et al., 2004). Although the current study investigated a similar cat population using similar methodology, the prevalence of *T. foetus* observed in purebred cats in

Germany was much lower. A possible explanation may be the difference in data collection. GOOKIN and colleagues (2004) collected faecal samples from cats present at the cat show which may have created a sampling bias towards diarrhoeic specimen. Cats may be unwilling to defecate in a stressful environment such as a cat show. Cats with large-bowel diarrhoea, however, have an increased urge to void and are not able to retain faeces over a long period of time. Therefore, these cats may be more likely to defecate at a cat show compared to cats with normal faecal consistency. Due to the strong association of *T. foetus* with large-bowel diarrhoea, the sampling of faeces voided only at the cat show may have led to an overestimation of trichomonosis among purebred cats. As faeces were not scored by GOOKIN and colleagues (2004), this bias cannot be ruled out.

On the other hand, the prevalence of *T. foetus* may have been underestimated in the present study. Recent use of antibiotics can lead to a decrease in the faecal shedding of trophozoites below the detection limit (GOOKIN et al., 1999; FOSTER et al., 2004; TOLBERT & GOOKIN, 2009). Also, due to the comparatively low sensitivity of current testing methods and suspected intermittent shedding of *T. foetus* trophozoites, a single negative test result can not conclusively rule out an infection (HALE et al., 2009; VERMEULEN, 2009). As the current study relied on testing of a single faecal sample and the possibility of antibiotic use within the previous two weeks was not excluded, the prevalence of *T. foetus* infection in purebred cats in Germany may actually be higher than the results indicate.

2. Detection methods

In this study, both faecal culture and faecal PCR were used to detect *T. foetus.* The pathogen was identified in 36 of 230 faecal samples. Of the 36 specimen, 29 tested positive using faecal culture and 28 tested positive by PCR amplification using diagnostic size variants from within the ITS1 region between the 18S rRNA and 5.8S rRNA subunits. These results are unexpected, as PCR is commonly thought to provide superior sensitivity compared to other diagnostic methods (GOOKIN et al., 2004; TOLBERT & GOOKIN, 2009; GALIAN et al., 2011). Thus, in a study by GOOKIN and colleagues (2004), sensitivity of single-tube

nested PCR from faeces was 94.4%, detecting 34 of 36 *T. foetus*-positive samples compared to faecal In Pouch^{TM} culture which detected only 20 of 36 positive samples. Yet faecal culture has exhibited greater sensitivity compared to faecal PCR in several studies (GOOKIN et al., 2002; STOCKDALE et al., 2009; VERMEULEN, 2009). In a study by STOCKDALE and colleagues (2009), only 12 of 17 culture-positive faecal samples tested positive for *T. foetus* on PCR. Similarly, in a recent Dutch study which used PCR of inoculated culture as a gold standard, faecal culture using In Pouch^{TM} had a calculated sensitivity of 52.2% compared to faecal PCR which had a sensitivity of only 43.5% (VERMEULEN, 2009). GOOKIN and colleagues (2002) found that faecal PCR amplification was not superior to faecal culturing upon examination of faecal samples of experimentally infected cats, with single-tube nested PCR yielding only 39% positive results. The most probable explanation for the comparatively low detection rate of the PCR assay is not a poor absolute detection limit but is rather most likely due to coextraction of PCR inhibitors during DNA extraction. Endogenous PCR inhibitors such as complex polysaccharides, bile salts, hemoglobin degradation products, phenolic compounds, and heavy metals can dramatically decrease the analytic sensitivity of faecal *T. foetus* PCR assays (GOOKIN et al., 2002; STAUFFER et al., 2008). Although the current study followed a specially modified DNA extraction protocol allowing for extendend incubation of extracted DNA with a higher concentration of proteinase K and additional elution, coextraction of faecal components which may have interfered with PCR performance can not be ruled out as no amplification controls were used for the detection of PCR inhibitors. The DNA extraction kit in the current study may have decreased the sensitivity of the PCR assay as compared to the PCR used by GOOKIN et al. (2004) due to a change of components in the extraction kit resulting in less sensitive and reproducible detection of *T. foetus* than previously reported (STAUFFER et al., 2008). To improve the detection rate of *T. foetus* in this study, both faecal culture and PCR from faeces were used because the combined sensitivity of both tests has been shown to be considerably higher than the sensitivity of either test alone (GOOKIN et al., 2002).

3. Clinical signs

The following clinical signs of gastrointestinal disease were evaluated in this study: faecal consistency, severity, and duration of diarrhoea if present, weight loss, signs of large bowel disease, specifically faeces accompanied by mucus and haematochezia, and faecal incontinence.

3.1. Diarrhoea

Various studies of both experimental and natural *T. foetus* infection have clearly established trichomonads as the causative agent of chronic large-bowel diarrhoea in cats (GOOKIN et al., 1999; GOOKIN et al., 2001; FOSTER et al., 2004; GOOKIN et al., 2004; STOCKDALE et al., 2009).

3.1.1. Prevalence of *T. foetus*-associated diarrhoea

Most studies on feline trichomonosis to date have either focused on detecting *T. foetus* only in cats with chronic diarrhoea or have screened cats for infection, but have not scored faecal samples (GOOKIN et al., 1999; FOSTER et al., 2004; GOOKIN et al., 2004; GUNN-MOORE et al., 2007; BISSETT et al., 2008; BURGENER et al., 2009; FREY et al., 2009; HOLLIDAY et al., 2009; VERMEULEN, 2009). Consequently, the association of feline *T. foetus* infection with clinical signs has rarely been investigated in a population of cats with and without gastrointestinal signs.

Several authors have suggested that asymptomatic *T. foetus* infection may be quite common (TOLBERT & GOOKIN, 2009; GRAY et al., 2010). Thus, in a recent investigation of 36 *T. foetus*-positive catteries in the USA, only 24.6% of infected 61 purebred cats had a history of clinical signs. *T. foetus* was not significantly associated with diarrhoea (GRAY et al., 2010).

In contrast, the current study demonstrated a strong association between *T. foetus* and diarrhoea. Infection correlated significantly with i) an abnormal faecal score on the day of the survey, ii) a history of diarrhoea in the sampled cat, and iii) a history of diarrhoea in the associated cattery in the past six months. Only 11.1% of 36 cats diagnosed with *T. foetus* had a normal faecal consistency on the day of the cat show, and diarrhoea correlated strongly with the detection of faecal trichomonads ($p < 0.001$). A history of diarrhoea in the past six months was documented in 61.1% (22/36) of *T. foetus*-positive cats and 69.6% (16/23) of

infected catteries. Infection with other enteroparasites was not correlated with diarrhoea. In a study by STOCKDALE and colleagues (2009), all 17 cats with *T. foetus* had diarrhoea at the time the faecal sample was taken. Of 104 infected cats recently surveyed in a retrospective study, 98.1% had a history of diarrhoea, 58.6% of which had exhibited clinical signs since adoption (XENOULIS et al., 2010a). Although VERMEULEN (2009) found no correlation in the outcome of PCR testing and faecal score in a longitudinal study of infected cats, it has been postulated that *T. foetus* may be easier to detect in diarrhoeic faeces. In the current study, this may have caused a detection bias toward symptomatic *T. foetus*-positive cats. Interestingly, a considerable percentage of infected cats had an abnormal faecal consistency on the day of the cat show that did not have a history of diarrhoea as reported by the owners. This finding is consistent with isolated bouts of diarrhoea triggered by stressful events often observed in cats with *T. foetus* (FOSTER et al., 2004).

3.1.2. Association of diarrhoea with age

A significant proportion of cats with trichomonosis may have complete resolution of *T. foetus*-associated diarrhoea over time but remain persistently infected (FOSTER et al., 2004). Therefore, it has been postulated that chronic asymptomatic *T. foetus* infection may be quite common particularly in older animals (FOSTER et al., 2004; XENOULIS et al., 2010b). These asymptomatic carriers may serve as a source of infection for other cats, contributing to the spread of *T. foetus* within the household (FOSTER et al., 2004; TOLBERT & GOOKIN, 2009). *T. foetus* was recently detected in six of 31 cats in Greece, all six of whom had normal faecal consistency. Five of these asymptomatic cats were adults over one year of age (XENOULIS et al., 2010b). In contrast, in the present study older infected cats commonly had diarrhoea, and age of infected cats was not significantly associated with clinical signs or severity of disease. However, only 11 of 36 infected cats were older than one year of age and only four of these cats were above three years of age. The low number of older *T. foetus*-positive cats and resulting low statistical power precluded testing of the hypothesis that older animals are less likely to suffer from *T. foetus*-associated diarrhoea.

3.1.3. Association of diarrhoea with enteroparasitic co-infections

While the multifactorial pathogenesis of clinical symptoms is well known in

giardiasis, an infection caused by a related flagellated protozoal parasite (PAYNE & ARTZER, 2009), studies of *T. foetus* in cats have not yet clarified the role of concurrent infection with other enteropathogens, endogenous microflora, environmental stress, and host immune status in the clinical manifestation of disease (GOOKIN et al., 1999; GOOKIN et al., 2001; STOCKDALE et al., 2009). Although enteric co-infections have frequently been documented in *T. foetus*-positive cats (GOOKIN et al., 1999; GOOKIN et al., 2001; GOOKIN et al., 2004; BURGENER et al., 2009; STOCKDALE et al., 2009), their association with clinical signs has not been well established. A study of experimental *T. foetus* infection observed more severe diarrhoea and increased shedding of trichomonads in four cats concurrently infected with *Cryptosporidium* spp. (GOOKIN et al., 2001). The current study of naturally infected cats, on the other hand, found no association between co-infection with other enteric parasites and severity of *T. foetus*-associated diarrhoea. Concurrent infections with *Giardia* spp. and coccidia were observed in 12 (33.3%) of 36 *T. foetus*-positive cats and had no effect on faecal consistency as determined by faecal score. Thus, this study found no evidence to support that coexisting enteric infections worsen clinical signs of feline trichomonosis.

3.2. Other clinical signs

Feline *T. foetus* infection was not associated with weight loss. This finding is not unexpected, as *T. foetus* infection is predominantly a large bowel disease in cats. Symptoms of small bowel disease such as weight loss are uncommon, and may occasionally be attributed to concurrent enteroparasitic infections such as giardiasis.

Interestingly, no association was observed between *T. foetus* infection and classic signs of large bowel disease. Thus, breeders reported faeces accompanied by mucous or fresh blood in only 35.3% of cases. In contrast, in a recent retrospective study of 104 *T. foetus*-positive cats living in North America, 58.7% of infected cats had mucous in their faeces and 45.5% of cats had hematochezia (XENOULIS et al., 2010a), indicating that these symptoms are commonly observed in *T. foetus*-positive cats. Why these symptoms were reported so seldom in association with *T. foetus* by breeders in this study is unclear. Perhaps faeces in litter boxes was not routinely inspected closely so that blood and mucous were missed. Alternatively, many infected cats in this study may have had chronic

recurrent signs of infection, as previously postulated. It is feasible that intermittent bouts of diarrhoea associated with *T. foetus* may not cause as severe inflammation of the large bowel as the acute phase of *T. foetus* infection and may, therefore, not be as commonly associated with mucous and fresh blood.

Faecal incontinence, observed in nearly 14.9% of infected cats by XENOULIS and coworkers (2010a), was observed in only two of 22 cats with *T. foetus*-associated diarrhoea in this study and was not associated with *T. foetus* infection. In fact, faecal dribbling was more commonly observed in diarrhoeic cats without *T. foetus* indicating that this symptom commonly considered a classic sign of severe *T. foetus* infection may not be as specific as previously assumed.

4. Determinants of infection

In the present study, risk factors for *T. foetus* infection were evaluated using a detailed epidemiological questionnaire that had to be filled out for each cat.

4.1. Signalment

In the past, several studies have suggested that *T. foetus* infection is primarily a disease of purebred cats and of young cats and kittens (GOOKIN et al., 1999; GUNN-MOORE et al., 2007; BURGENER et al., 2009; STOCKDALE et al., 2009).

4.1.1. Age

In agreement with current views, this study was able to show a significant association of *T. foetus* with young age. Twenty-five (69.4%) of 36 infected cats were aged one year and younger, and prevalence of disease decreased with age. Thus, of the 28 cats older than five years sampled in this study, only one cat tested positive for trichomonosis. However, a recent investigation in a rescue colony in Italy found that 66.7% of 24 infected cats were over one year of age (HOLLIDAY et al., 2009). Similarly, all six cats diagnosed with *T. foetus* in a Greek study were over one year of age (XENOULIS et al., 2010b). While this clearly indicates that older cats are also at risk for disease, an age-dependent susceptibility for *T. foetus* infection may exist in that younger cats may be more vulnerable to disease due to an immature immune system (GOOKIN et al., 1999).

Alternatively, the high prevalence of trichomonosis among kittens and young cats may reflect the time point at which transmission of *T. foetus* within a cattery is most likely to occur.

4.1.2. Breed

While several breeds such as Maine Coon and British Shorthair cats were overrepresented in the present study, only Norwegian Forest (NFO) cats and NFO catteries were at a significantly higher risk of *T. foetus* infection than other breeds. Thus, trichomonosis was identified in nearly 66.7% (10/15) of NFO cats and 87.5% (7/8) of NFO catteries. Detailed questioning of infected catteries throughout Germany identified no association between infected NFO cats regarding recent ancestry or breeding programs. Because of the high prevalence of *T. foetus* among purebred cats, the possibility that some breeds may have a genetic predisposition for trichomonosis has been repeatedly discussed (GUNN-MOORE et al., 2007; STOCKDALE et al., 2009). However, while two studies of diarrhoeic cats in Switzerland identified *T. foetus* in NFO cats, this breed was not overrepresented among infected cats (BURGENER et al., 2009). Furthermore, in a recent German study only 22.2% of 73 faecal samples of NFO cats submitted to a German reference laboratory tested positive for *T. foetus* and the NFO breed was not significantly associated with infection (GALIAN et al., 2011). Therefore, while a genetic predisposition of NFO cats in Germany cannot be ruled out with certainty at this time, it does not appear likely and other possible associations among the infected NFO cats in the present study such as country of acquisition of cats or similar management within these NFO catteries should be more closely investigated.

4.2. Co-infection with other enteroparasites

Enteric parasites other than *T. foetus* were identified in 49 of 230 faecal samples. Co-infections were identified in 14 of 36 *T. foetus*-positive cats. Of these, one cat was coinfected with *Cryptosporidium* spp., seven were coinfected with *Giardia* spp. only, and three cats with *Isospora* spp. only. Three cats with *T. foetus* tested positive for both *Giardia* spp. and *Isospora* spp.. No association was found between *T. foetus* infection and either *Giardia* or *Isospora* infection. The small number of faecal specimen positive for both *T. foetus* and *Cryptosporidium* species (n = 1) precluded analysis of feline co-infections with these two parasites.

4.3. Environmental factors

Potential environmental risk factors that were investigated in this study included diet, water source, indoor *versus* outdoor housing, number of cats in household, housing density measured in square metres per cat, litter box management, presence of other pets in household, contact to cats outside of household, and proximity to livestock.

4.3.1. Housing density

The high prevalence of *T. foetus* infection observed in purebred cats as compared to pet and feral cats is thought to be due to differences in living conditions (GOOKIN et al., 1999; GOOKIN et al., 2004; GRAY et al., 2010). Indoor high density multi-cat housing, as commonly found in catteries and shelters, has come to be regarded as a key risk factor for feline trichomonosis. Square feet of facility per cat approached significance in a study of *T. foetus* in purebred cats living in catteries ($p = 0.056$) (GOOKIN et al., 2004). Although the exact mode of transmission of *T. foetus* is unknown, crowding is thought to increase infection pressure due to an increased risk of faecal-oral contact (GOOKIN et al., 2004; TOLBERT & GOOKIN, 2009).

Unexpectedly, the current study was not able to identify an association between *T. foetus* infection and housing density. Purebred catteries surveyed by GOOKIN and colleagues (2004) contained a median number of 16 cats with a range of one to 59 cats per household and a median of 71.4 square feet of facility per cat in infected catteries. In contrast, the housing density of cats in catteries documented in the current study was much lower. Sampled catteries contained a median number of seven cats with a range of one to 33 cats per household and a median of 129 square feet of facility per cat in catteries testing positive for *T. foetus.* Perhaps the housing density observed in this study was not high enough to act as a risk factor for disease. On the other hand, it is also possible that survey questions asking for the number of cats per household and square meters per facility were not answered honestly, as animal protection rights in Germany frown upon high density housing situations. For fear of repercussions, cat owners may have been reluctant to provide the veterinarian distributing the surveys for this study with accurate information on the total number of cats and size of their facility. Finally, the unexpected results regarding housing density may also be attributed to the questionnaire used in this study as the survey did not differentiate

between adult cats and kittens. Data analysis revealed that while some owners only listed the number of adult cats in their cattery, other owners also included the number of kittens. This impaired evaluation of the permanent number of cats per household and may have skewed results of the calculated housing density.

4.3.2. Management of litter boxes

No correlation was observed between *T. foetus* infection and the ratio of cats to litter boxes. Similarly, GOOKIN and coworkers (2004) identified no association with litter box management or the type of litter used. These results are unexpected considering the presumed environmental fragility of *T. foetus* trophozoites. Because trichomonads do not form environmentally stable cysts, only freshly voided faeces were thought to contain infectious organisms (GOOKIN et al., 2004), so that shared litter boxes were implicated in the faecal-oral spread of *T. foetus* (TOLBERT & GOOKIN, 2009). Recently, however, an experimental study showed that at room temperature *T. foetus* is more resilient in cat faeces than previously assumed. In moist faeces, the organism can survive for at least seven days (HALE et al., 2009), indicating that faecal-oral spread may not require contact with a fresh faecal specimen. Thus, it is conceivable that transmission of *T. foetus* is not limited to immediate oral contact with faeces in the litter box or to ingestion during grooming of freshly soiled fur. Previously ruled out as a possible risk factor, environmental contamination with moist faeces, especially diarrhoea, from infected cats may contribute to the spread of *T. foetus* and may require more stringent sanitation measures than previously assumed. Also, a recent Australian study found that *T. foetus* can survive passage through the alimentary tract of two common garden slugs. This study also demonstrated that *T. foetus* is viable in wet cat food for five days, thus indicating that *T. foetus* may also be spread indirectly (VAN DER SAAG et al., 2010). Whether this type of indirect transmission plays a role in the German catteries examined in the current study is unclear, but seems unlikely as the majority of cats were indoor cats without access to slugs. A final explanation for the lack of association between *T. foetus* infection and litter box management may be the time point of transmission. As discussed above, prevalence of infection is highest among young cats, and kittens are also commonly infected. Thus, it is plausible that the risk of faecal-oral transfer is greatest between an infected queen and her kittens and, subsequently, among kittens within that litter. In this case, the management of the

queening box and kittening pen would take precedence over management of litter boxes used by adult cats in controlling the spread of disease within a cattery. As epidemiological information concerning transmission from queen to kittens is lacking, the incidence of infection among pre-weaning kittens should be thoroughly investigated.

4.3.3. Proximity to livestock

The source of *T. foetus* infection in cats remains undetermined. As *T. foetus* was recognized in cattle long before becoming known as a pathogen in cats, a question of great interest has been whether cows with *T. foetus* are a potential source of infection for cats and *vice versa*. In agreement with the study by GOOKIN and colleagues (2004), the epidemiological survey used in this study did not identify an association of *T. foetus* infection with any direct or indirect contact to livestock. Recent experimental studies investigating cross-species transmission of bovine and feline *T. foetus* isolates found an observable difference in the infectivity of both isolates in cats (STOCKDALE et al., 2007; STOCKDALE et al., 2008). Furthermore, molecular characterization of multiple feline *T. foetus* isolates as compared to bovine *T. foetus* isolates using bidirectional sequencing has identified significant genetic differences between isolates from cattle and domestic cats (SLAPETA et al., 2010). Supported by the current study which found no association between *T. foetus*-positive catteries and proximity to livestock or agricultural facilities, the findings above suggest that *T. foetus* isolates are host-adapted and that cattle do not play an important role in the spread of feline trichomonosis.

5. Conclusion

In conclusion, this study found a high prevalence of *T. foetus* infection in purebred cats throughout Germany. None of the cats participating in this study had ever been tested for *T. foetus*, and only one cattery owner was aware of *T. foetus* as an infectious agent in cats, confirming that feline *T. foetus* is a newly emerging pathogen in Germany. Clinical disease was very common indicating that *T. foetus* infection should be considered an important differential diagnosis for chronic diarrhoea in purebred cats in Germany, especially in young animals under one year of age. However, no correlation was observed between the age of

infected cats and the severity of diarrhoea, indicating that cats of all age groups may develop clinical signs. Concurrent infection with enteric parasites was not correlated with prevalence or severity *T. foetus*-associated diarrhoea suggesting that *T. foetus* infection alone is sufficient to cause significant clinical disease. No evidence was found to support the notion that clinical signs of *T. foetus* infection are exacerbated by enteric co-infections.

Unexpectedly, *T. foetus* infection was not correlated with either housing density or ratio of cats to litter boxes, nor any other environmental factors including diet, water source, direct and indirect contact to livestock or other pets, and proximity to agricultural facilities. No evidence was found to support the epidemiologic role of asymptomatic carriers in the spread of disease within multi-cat households.

V. SUMMARY

Tritrichomonas foetus (*T. foetus*), a flagellated protozoon causing bovine venereal trichomoniasis, has recently been recognized as an important feline enteric pathogen associated with chronic-intermittent large bowel diarrhoea in domestic cats. Although cases of feline *T. foetus* have been reported in cats with diarrhoea in several countries throughout Europe including Germany, true prevalence and geographic range of *T. foetus* infection in cats in Germany are yet unknown. The purpose of this study was (i) to determine the prevalence of *T. foetus* in purebred cats in catteries throughout Germany, (ii) to evaluate the frequency of clinical symptoms and investigate the role of co-infection on the severity of disease, and (iii) to identify determinants of infection.

In this prospective study, freshly voided faecal samples of 230 cats representing 124 catteries were collected at five regional cat shows throughout Germany. Upon collection, all faecal specimens were scored numerically using a continuous modified faecal scoring system based on faecal consistency, with a scale of 1, representing liquid diarrhoea, to 4, representing firm faeces. The cats' owners were asked to fill out an epidemiological questionnaire providing detailed information on signalment, living conditions, and medical history. Faecal samples were examined for *T. foetus via* culture in InPouch^{TM} medium and PCR amplification using conserved trichomonad primers specific for *T. foetus* diagnostic size fragments. Faeces were also analyzed for *Giardia* cysts, coccidia, and helminth ova using zinc sulphate centrifugation flotation and a monoclonal microplate immunoassay for *Giardia spp.* and *Cryptosporidium* spp..

The prevalence of *T. foetus* infection was 15.7% (36/230) among individual cats and 18.5% (23/124) among catteries. An abnormal faecal score, observed in 63.9% of *T. foetus*-positive cats, and a history of diarrhoea in the past six months, reported in 61.1% of infected cats, correlated significantly with *T. foetus* infection. A history of diarrhoea among cats living in infected households, which was documented in 69.6% of *T. foetus*-positive catteries, was also significantly associated with *T. foetus* infection. Firm faeces, corresponding to a faecal score of 4, were observed in only four (11.1%) of 36 infected cats. Among the enteroparasites detected in this study, *T. foetus* was the only pathogen associated

with diarrhoea. Co-infections with other intestinal parasites were detected in 13 of 36 *T. foetus*-positive cats and were not associated with either an abnormal faecal consistency or a history of diarrhoea. The age of infected cats ranged from three weeks to 7.2 years with a median age of one year, and the prevalence of infection correlated significantly with young age ≤ 1 year. Of the 11 of 25 breeds in which *T. foetus* was identified, Norwegian Forest (NFO) cats were significantly overrepresented. Thus, 66.7% (10/15) of NFO cats and 87.5% (7/8) of NFO catteries tested positive for *T. foetus*. No association was found between *T. foetus* infection and any environmental factors, including proximity to livestock, type of diet, water source, and number of cats per household.

In conclusion, this study demonstrates a high prevalence of feline *T. foetus* in catteries throughout Germany. Clinical disease was very common, indicating that *T. foetus* should be considered an important differential diagnosis for chronic large-bowel diarrhoea in young purebred cats. The higher prevalence of an abnormal faecal consistency on the day of the cat show as compared to the recent history of diarrhoea in infected cats supports the notion that recurrent bouts of *T. foetus*-associated diarrhoea may be brought on by environmental stress. Importantly, co-infection with other enteroparasites had no effect on either prevalence or severity of diarrhoea, indicating that *T. foetus* alone is sufficient to cause clinical disease.

VI. ZUSAMMENFASSUNG

Der Protozoe *Tritrichomonas foetus* (*T. foetus*), Auslöser der bovinen Deckseuche, wurde kürzlich auch bei Hauskatzen entdeckt und gewinnt seitdem bei dieser Spezies als Erreger von chronisch-rezidivierendem Dickdarmdurchfall zunehmend an Bedeutung. Obwohl über Fälle von felinen *T. foetus* Infektionen bereits in mehreren Ländern Europas inklusive Deutschland berichtet wurde, sind Prävalenz und geographische Ausbreitung des Erregers in Deutschland noch unbekannt. Ziele dieser Studie waren daher (1) die Prävalenz von *T. foetus* bei Rassekatzen in Deutschland zu bestimmen, (2) die Häufigkeit symptomatischer Infektionen und den Einfluss parasitärer Koinfektionen auf den Schweregrad der Symptome zu untersuchen und (3) prädisponierende Faktoren für einen Trichomonadenbefall zu identifizieren.

In dieser prospektiven Studie wurden frische Kotproben von 230 Rassekatzen aus insgesamt 124 Katzenzuchten auf fünf Katzenausstellungen in Deutschland gesammelt. Die Kotkonsistenz wurde numerisch anhand eines modifizierten Punktesystems mit einer durchgehenden Skala von 1 (wässrigem Durchfall) bis 4 (festem Kot) bewertet. Detaillierte Informationen zu Signalement, Haltungsbedingungen und Krankengeschichte wurden mithilfe eines epidemiologischen Fragebogens erhoben. Kotproben wurden mittels InPouch™ Kultur und *via* PCR unter Verwendung konservierter speziesspezifischer Primer auf *T. foetus* untersucht. Giardien-Zysten, Kokzidien und Helmintheneier im Kot wurden mit dem Zinksulfat-Zentrifugation-Flotationsverfahren ermittelt. Zum Nachweis von *Giardia*-spp-. und *Cryptosporidium*-spp.-Koproantigen wurden monoklonale Immunoassays eingesetzt.

Eine *T.-foetus*-Infektion wurde bei 15,7 % (36/230) der Katzen und 18,5 % (23/124) der Katzenzuchten nachgewiesen. Sowohl eine anormale Kotkonsistenz, bei 63.9 % der *T.-foetus*-positiven Katzen dokumentiert, als auch vorberichtlicher Durchfall, laut epidemiologischen Fragebogen bei 61.1 % infizierter Katzen beobachtet, waren signifikant mit einer *T.-foetus*-Infektion assoziiert. Vorberichtlicher Durchfall wurde bei Katzen in 69.6 % der *T.-foetus*-positiven Zuchten beobachtet und korrelierte ebenfalls signifikant mit einem Trichomonadenbefall. Eine feste Kotkonsistenz war nur bei vier von 36 infizierten

Katzen zu beobachten. Von den in dieser Studie untersuchten Enteropathogenen war ausschließlich *T. foetus* mit Durchfall assoziiert. Koinfektionen mit anderen intestinalen Parasiten wurden bei 13 von 36 *T.-foetus*-positiven Katzen nachgewiesen. Sie korrelierten weder mit einer anormalen Kotkonsistenz noch vorberichtlichen Durchfall. Das Alter *T.-foetus*-infizierter Katzen lag zwischen drei Wochen und 7,2 Jahren, und betrug im Median ein Jahr. Bei Katzen ≤ 1 Jahr wurde der Erreger signifikant häufiger diagnostiziert. *T. foetus* wurde bei 11 von 25 verschiedenen Katzenrassen nachgewiesen. Norwegische Waldkatzen (NFO) und NFO-Zuchten waren mit einer Prävalenz von 66,7 % (10/15) bzw. 87,5 % (7/8) signifikant häufiger *T.-foetus*-positiv als andere Rassen. Es wurde keine Korrelation zwischen *T.-foetus*-Infektion und untersuchten Umweltfaktoren, unter anderem Kontakt zu Nutztieren, Ernährung, Trinkwasserquelle und Bestandsdichte, gefunden.

Zusammenfassend wurde in dieser Studie eine hohe Prävalenz von *T. foetus* bei deutschen Rassekatzen nachgewiesen. Die Mehrheit der infizierten Katzen hatte klinische Symptome, so dass ein Trichomonadenbefall vor allem bei jungen Rassekatzen als wichtige Differentialdiagnose chronischen Dickdarmdurchfalls betrachtet werden sollte. Eine abnormale Kotkonsistenz am Tag der Katzenausstellung trat bei *T.-foetus*-positiven Katzen ohne vorberichtlichen Durchfall signifikant häufiger auf als bei *T.-foetus*-negativen Katzen. Dies untermauert die Rolle von Umweltstress als Auslöser der für eine *T.-foetus*-Infektion charakteristischen intermittierenden Durchfallschübe. Parasitäre Koinfektionen hatten weder Einfluss auf die Häufigkeit noch den Schweregrad von *T.-foetus*-assoziiertem Durchfall, welches die Bedeutung von *T. foetus* als primären Durchfallerreger unterstreicht.

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