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PCR analyses by IDEXX

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*Tritrichomonas foetus* Infection in Domestic Cats (IDEXX data)

Final Year Research Project Report

(60 credits)

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**Abstract**

*Tritrichomonas foetus* (*T. foetus*) is a single-celled protozoan that is well known as the causative agent of venereal trichomonosis in cattle (Emmerson, 1932). Recently it has also been recognized 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 (Riedmuller. 1928; Yule *et al*., 1989), in cats, *T. foetus* causes large-bowel disease and is associated with chronic diarrhoea (Gookin et al. 1999; Yaeger and Gookin. 2005).

Faecal samples from 19,111 domestic cats from the UK were submitted to IDEXX Laboratory, where they were analysed for *Tritrichomonas foetus* using polymerase chain reaction (PCR) technology, between February 2010 and October 2017. The aim of this study was to determine *Tritrichomonas foetus* prevalence and identify the risk factors associated with infection, mainly age, breed, neutered status, season and geographical location. The annual incidence over the study period was also assessed.

The overall UK prevalence of *T. foetus* between February 2010 and October 2017 was 14.6% (2792/19111). Age, breed, season, year, gender and geographical location were found to be predispositions of *T.foetus* infection; neutered status had no significant association. The results of this investigation indicated that *T.foetus* infection is an emerging disease, a previously unrecognized cause of diarrhoea in cats in the UK. It is therefore an important diagnosis to consider when investigating cats showing clinical signs, especially in cats that are under 1 year of age.

**Lay Summary**

*Tritrichomonas foetus* (*T. foetus*) is a species of parasite, well known for being a pathogen of the bovine reproductive tract, leading to infertility and abortion in cows. As well as, more recently, also being a pathogen of the feline gastrointestinal tract causing large bowel disease and chronic diarrhoea in domestic cats.

Using a large study population from a national database, IDEXX, this report had, as an objective, to report the annual incidence of *T.foetus* in the UK, from February 2010 to October 2017, while identifying the risk factors related to this parasitic infection in cats, such as age, breed, gender, neutered status, season, year and geographical location.

The overall UK incidence of *T. foetus* infection was 14.6% (2792/19111). Age, breed, season, year, gender and geographical location were found to affect the likelihood of *T.foetus* infection in cats.

**Table of Contents**

1. **Abstract**  3
2. **Lay Summary**  4
3. **Introduction** 7
   1. Morphology and Life Cycle 7
   2. Pathogenicity 9
   3. Detection 10
   4. Determinants of Infection 11
   5. Treatment and Prognosis 11
   6. Aims 12
   7. Hypothesis 12
4. **Materials and Methods** 13
   1. Study Design 13
   2. Laboratory Techniques 13
   3. Data and Statistical Analysis 13
5. **Results** 15
   1. Prevalence 15
   2. Age 15
   3. Breed 16
   4. Gender 17
   5. Neutered Status 18
   6. Annual Incidence 18
   7. Season 19
   8. Geographical Origin 20
6. **Discussion** 21
   1. Prevalence in the UK 21
   2. Determinants of Infection 21
      1. Age 21
      2. Breed 22
      3. Gender 23
      4. Neutered Status 23
      5. Annual Incidence 24
      6. Season 24
      7. Geographical Origin 24
   3. Limitations of the Study 25
   4. Conclusion 25
7. **Acknowledgements** 27
8. **References** 28
9. **Appendix I** 35
10. **Appendix II** 37

**Introduction**

*Tritrichomonas foetus (T.foetus)* is a species of protozoan parasite known traditionally for being a pathogen of the bovine reproductive tract as well as, more recently, of the feline gastrointestinal tract (BonDurant. 1997).

First described in cattle as a venereal disease characterized by early to midgestation pregnancy loss, endometritis, pyometra and infertility (BonDurant. 1997). *T foetus* is sexually transmitted among cattle from infected, asymptomatic bulls to heifers or cows at the time of coitus or by artificial insemination using contaminated seminal fluid (Fellesein, 1999).

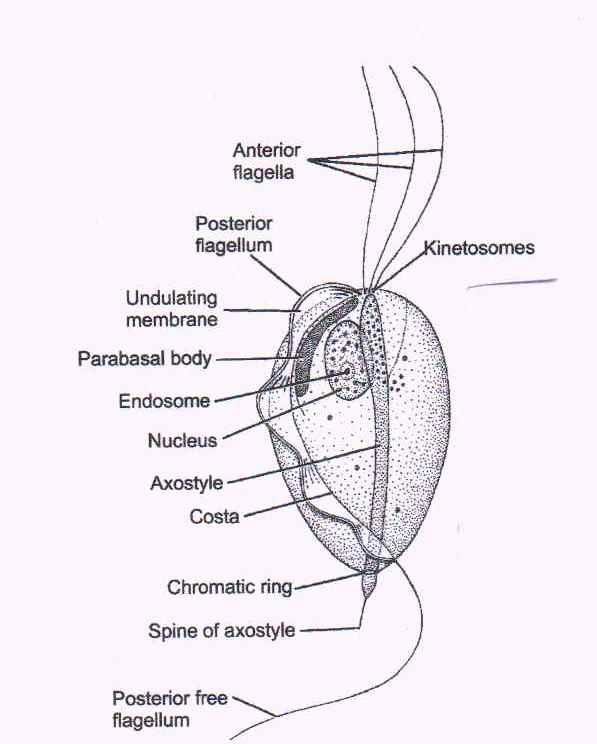
Feline *T.foetus* is currently recognized as a primary cause of large bowel disease and chronic diarrhoea in domestic cats (Gookin *et al*. 1999; Gunn-Moore *et al.* 2007). Infection occurs through direct faecal-oral transmission, with *T. foetus* colonising the ileum, caecum, and colon where it can cause lymphocytic, plasmacytic inflammation and mild to moderate colitis, (Gookin *et al.* 2001; Yaeger and Gookin. 2005)

**Morphology and Life Cycle**

Trichomonads are anaerobic, single-celled, flagellated protozoans (Kulda *et al.* 1999). *Tritrichomonas foetus* is the genus *Tritrichomonas* belongs to the order Tritrichomonadida in the Kingdom *Protocitista* (Levine *et al.* 1980).

The parasite has a pleomorphic, spindle-shaped body approximately 10-25 μm in length and 3-12 μm in width, with a single nucleus situated at the anterior end of the cell body (Figure 1) (Riedmuller. 1928; Wenrich and Emmerson. 1933). The cytoskeleton consists of an axostyle, pelta, and costa. The axostyle supports the cell contributing to karyokinesis and prolongs beyond the length of the cell body (Levine *et al.* 1985; Benchimol. 2004)

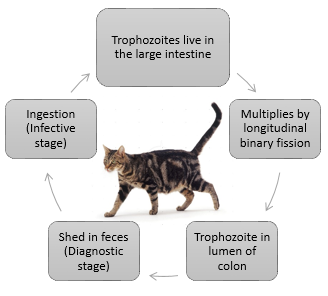
Organisms belonging to the genus *Tritrichomonas* have three anterior flagella and a posterior flagellum, which constitutes the marginal filament of the nearly full-length undulating membrane (Warton and Honigberg. 1979). Movements of the recurrent flagellum are transmitted to the undulating membrane which then acts as an associate locomotory organ and is reinforced by the costa, a feature characteristic of trichomonads (Benchimol 2004). The flagella originate from basal bodies that are made up of contractile centrin fibres which allow internalization of flagella during pseudocyst formation. (Stockdale. 2008).



**Figure 1.** *Tritrichomonas foetus* morphology (Roberts and Janovy. 2009)

Trichomonads are anaerobic meaning that is adapted for living in a mucous membrane-lined, anaerobic-to-microaerophilic, non-sterile organ cavities such as the gastrointestinal and reproductive tracts, in this case, the colon (Bornside *et al.* 1976; Gookin, Hanrahan and Levy. 2017).

They have a simple life cycle (Figure 2) dictated by the trophozoite form. Pseudocyst formation has also been described in intestinal trichomonads, only when is considered a degenerate cell form (Boggild *et al*. 2002; Pereira-Neves *et al*. 2003).



**Figure 2.** *Tritrichomonas foetus* life cycle (Schematics adapted from Veterian Key. 2016)

*T.foetus* reproduce via binary longitudinal fission and replication occurs through closed mitosis under contribution of the spindle, flagella, and axostyle (Ribeiro *et al*. 2002; Wenrich and Emmerson. 1933). This is considered to be a primitive version of mitosis as the spindle is extranuclear, the nuclear envelope does not break down and the Golgi apparatus does not divide (Benchimol, 2004). *T.foetus* has five chromosomes that become condensed during pre-mitosis and persists through mitosis. (Ribeiro *et al*. 2002). The nucleolus is duplicated during pre-mitosis and is observable during mitosis. The cell division is presented by duplication of the skeletal structures such as the basal bodies, flagella, and cytoskeleton throughout the premeiotic phase. (Benchimol *et al*. 2001).

Pseudocysts are also able to undergo division. Nonetheless, the process differs from the trophozoites in that under circumstances of environmental stress, it performs nuclear and amastigote division without corresponding cytoplasmic division, thus creating a polymastigote cell (Pereira-Neves *et al.* 2003). Is reversible to the trophozoite form upon stable environmental conditions.

**Pathogenicity**

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

Feline tritrichomonosis pathogenesis is limited and not well understood. It is not yet clear whether *T. foetus* is sufficient to cause a 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).

Trichomonads reportedly lack true mitochondria and in its place have primitive organelles, hydrogenosomes that enable anaerobic metabolism (Martin 2005) this adaptation permits them to live as lumen dwellers in oxygen-poor mucosal environments such as the gastrointestinal tracts of their hosts (Schewebkle JR; Burgess D. 2004). Studies that explore the influence of colonic microenvironment of *T.foetus* in cats could provide fundamental insight into disease pathogenesis, targets for supporting colonization resistance, or novel approached to restore normal colonic function (Tolbert and Gookin. 2006). However, at this time, such studies have not been published.

*T.foetus* has been determined to colonize the lumen of the ileum, cecum, colon, and rectum after experimental orogastric infection of cats (Gookin *et al*. 2001; Stockdale *et al*. 2008).

In colonic mucosal biopsy specimens from cats with *T.foetus* infection, trichomonads are reported to be adhering to the intestinal epithelium causing alteration to endogenous bacteria flora, releasing cytotoxins, crypt epithelial hypertrophy and crypt micro-abscesses in more than 80% of cases (Gookin *et al.* 2009). Eosinophilic inflammation is occasionally observed but is not considered a common feature of *T.foetus* infection (Yager and Gookin. 2005; Schrey *et al.* 2009). This is believed to represent a critical role in establishing colonization and mediating host pathogenicity (Petrin D *et al*. 1998).

The interaction between trichomonads and the colonic bacteria has been discussed as a potential pathogenic mechanism (Foster *et al*. 2004; Payne and Artzer 2009). The bacterial host flora is recognized to be essential *to T.foetus* infection as it allows it to obtain essential nutrients (Gookin *et al.* 2009).

**Detection**

Diagnosis of definite *T.foetus* infection relies on confirming the presence of the protozoal organism in the cat. Another diagnosis may reveal regional lymphadenopathy and corrugation of the large bowel and abdominal ultrasonography. Colon-biopsies can reveal non-specific changes with mild to severe inflammation due to infiltration of lymphocytes and/or plasma cells.

Trichomonads are not detected on routine faecal analysis, therefore the diagnosis of *T.foetus* infection usually requires more precise procedures. A diagnostic can be attained by direct microscopy of faecal smears (14.7% sensitivity) - least sensitive method of *T.foetus* diagnosis; however, the level of detection can be enhanced using parasite culture, commercially available InPouchTM (58.8% sensitivity) (Gookin *et al.* 2002); polymerase chain reaction (PCR) amplifying *T.foetus* ribosomal DNA provides the highest level of sensitivity detecting both live and dead organisms (Gookin *et al*. 2002); trichomonads may also be detected on histopathology (Yager and Gookin, 2005).

No current test can be 100% reliable for *T.foetus* infection in cats. If TF is diagnosed, using one of the methods described above, it is very likely that the cat is infected, nonetheless, a negative result does not completely rule out infection (Gunn-Moore and Tennant. 2007). In these cases, the likelihood of an accurate diagnosis can be increased by using the most sensitive method and repeating the test if the cat continues with clinical signs of diarrhoea (Gunn-Moore and Lalor, 2011).

*Tritrichomonas foetus* diagnostic methods rely on the presence of the parasite in the faecal samples, while the alternative method for collecting samples comprises of a colonic faecal flush. This method involves the use of a soft-rubber catheter that is inserted into the proximal part of the descending colon (10~cms), with a 10ml syringe containing warmed (60-75°C) sterile saline attached. The resulting solution is then used for smear examination, culture or polymerase chain reaction (PCR) (Gunn-Moore and Lalor, 2011).In recent years, faecal PCR has been recommended as the primary diagnostic assay for TF infection as it is far more sensitive than the other methods (Gookin, *et al.* 2004).

**Determinants of Infection**

The majority of studies demonstrate multi-cat households, notably in pedigree cats of a young age to be most predisposed to infection with no gender tendency (Burgener *et al.* 2009; Gookin *et al*. 1999; Gookin *et al.* 2004; Gunn-Moore *et al.* 2007; Stockdale *et al*. 2009). Suggested reasons include underdeveloped immunity of kittens and an increased likelihood of faecal-oral transmission (Gookin et al. 2009; Tynses *et al.* 2011).

Husbandry circumstances rather than genetic predisposition are believed to be accounted for the high prevalence amongst pedigree cats despite them being over-represented in some studies. (Gookin *et al*.2004; Kuehner *et al*. 2011; Profizi *et al.*2013).Nonetheless, non-pedigree cats and rescued cats in shelters can also be affected so it should not be considered a disease of pedigree breeds. Under 1 year of age but has also been reported in older cats (Holliday, *et al*. 2009; Stockdale *et al* 2009).

Furthermore, numerous studies have reported co-infections with *Giardia*, though no significant association with *T.foetus* infection has been found (Gookin *et al.*2004;Bisset *et al.*2009; Burgener *et a*l 2009;; Kuehner *et al.*2011).

**Treatment and Prognosis**

*T.foetus* infection has been proved to be unresponsive to fenbendazole and enrofloxacin with clinical signs persisting after treatment has worn-out (Gookin *et al* 1999; Mardell and Sparkes 2006). Provision of a highly digestible diet to affect cats can help control clinical signs and is an important aspect of treatment.

The treatment of choice is Ronidazole (Protexin Pro-Kolin Enterogenic™) (30mg/kg PO q24hours for 14 days) which is a nitroimidazole, related to metronidazole, and is used to treat *T.foetus* in pigeons has also been used to treat *T.foetus* in cats (Gookin et al. 2006). However, is currently an unlicensed drug used in cats as it can cause unwanted side effects such as neurotoxicity (Gookin *et al*.2006; Rosado *et al* 2007; Papich *et al.* 2013). This drug is known to resolve diarrhoea spontaneously which leads to treating cats that are showing clinical signs and are positive on faecal smears (Tolbert and Gookin. 2009).

Prognosis is commonly positive and some cats overcome infection without treatment nevertheless it can take up to two years for clinical signs to resolve (Foster *et al .*2004).

**Aims and Objectives**

The aim of this study was to highlight the importance of *T.foetus* as an emerging disease within the UK and assist veterinary surgeons considering differentials for feline large bowel diarrhoea.

Using a large study population from a national database, IDEXX, the objectives of this study were to (i) establish the overall prevalence of *T. foetus* in UK domestic cats between 2010 and 2017; (ii) report the annual incidence of *T. foetus* during the study period; and (iii) identify risk factors associated with infection, including age, breed, neutered status, gender, season, year and geographical location.

**Hypothesis**

The hypothesis of this project was that the overall prevalence of *T.foetus* in the United Kingdom would have increased throughout the years, with younger cats, entire cats and pedigree breeds more likely to suffer from infection, as well as to be seen an increase of infection during colder seasons (Winter and Autumn). Geographical location and gender were hypothesized to have no association with *T. foetus* infection. It was also hypothesized that the overall UK prevalence was low (<15%).

**Materials and Methods**

**Study Design and Population**

This was a retrospective study performed using data collected by IDEXX Laboratory in Wetherby, Yorkshire between February 2010 and October 2017. The database consisted of 19111 faecal samples analysed for *T.foetus* infection from cats within the UK. Further information regarding the breed, age, gender, neutered status, geographical location were also available for each case included in the dataset.

**Laboratory Techniques**

Between February of 2010 and October of 2017, 19111 faecal samples were sent to IDEXX from across the United Kingdom and screened for *Tritrchomonas* infection using PCR.

**Data and Statistical Analysis**

Data were examined using Microsoft Excel version 2016. Cases were removed if the analysis was inconclusive due to incorrect laboratory submission or to insufficient sample size.

Age was primarily converted into decimals in order to distinguish separate groups of cats under the age of a year. Age was subsequently categorised into five sample groups: 0-0.5 ( from 0 to 6 month old cats); 0.5-1 (from 6 month to a year old cat); 1-4 (from 1 year to 4 year old cats); 4-8 (from 4 to 8 year old cats) and <8 (from 8 to 20). Information regarding was available for 17396 samples (91%) ranging from 0.08 to 20 years old (median age: 2 years).

Of the 17396 samples, 25% (4327/17396) were classified into the 0-0.5 age gap group, 15% (2834/17396) were classified into the 0.5-1-year-old group, 23% (3927/17396) were classified into the 1-4-year-old group, 15% (2566/17396) were classified into the 4-8-year-old group and 22% (3742/17396) were classified into the <8-year-old group.

Samples were also categorised as male and female and further classified into entire and neutered cats. Information for gender was available for 17733 (93%) samples of which 46% (8158/1733) were female and 54% (9575/17733) were male.

From the records, 15050 (79%) samples had information regarding the neutered status of which 13% (2030/15050) were entire females, 32% (4782/15050) were neutered females, 13% (1962/15050) were entire males and 42% (6276/15050) were neutered males.

Collapsing the variables into the categories entire and neutered cats, 27% (3992/15050) were entire and 74% (11058/15050) were neutered cats.

In addition, samples were also grouped into different breeds (n=35) and later categorised as pedigree or non-pedigree (domestic short- or long-hair) cats. Information for breeds was available for 16680 (87%) samples of which 57% (9507/16680) were non-pedigree and 43% (7173/16680) pedigree.

Postcodes from referring veterinary practices were assigned into 122 districts and then later combined into 13 regions for analysis. Of the 19111 samples, 98% (18746/19111) had information regarding the geographical location of the sample of which 122 were recognised (see Appendix II).

Regarding the year samples were submitted, 6% (1160/19111) were analysed in 2010, 10% (1912/19111) were analysed in 2011, 13% (2501/19111) were analysed in 2012, 15% (2787/19111) were analysed in 2013, 15% (2956/19111) were analysed in 2014, 15% (2814/19111) were analysed in 2015, 15% (2809/19111) were analysed in 2016 and 11% (2172/19111) were analysed in 2017.

This was subsequently combined into the following seasons: Winter (December to February), Spring (March to May), Summer (June to August) and Autumn (September to November) in order to investigate the incidence of *T.foetus* during different weather conditions.

Of the 19111 samples on the database, 24% (4639/19111) were analysed during Winter, 22% (4268/19111) were analysed during Spring, 24% (4633/19111) were analysed during Summer and 29% (5571/19111) during Autumn.

Statistical analysis was conducted using Prism version 7.0. The categorical variables: age, breed, gender, neutered status, geographical location and year of the sample were analysed individually using Chi-squared tests, with the significance level set at P<0.05.

**Results**

**Prevalence**

The overall UK prevalence of feline *T.foetus* between February 2010 and October 2017 was 14.6% (2797/19111).

**Age**

The age of *T.foetus*-positive cats ranged from 0.08 to 19 years old (median age: 1.8 years) while the age of *T.foetus*-negative cats ranges from 0.08 to 20 years old (median age: 2 years).

The prevalence of *T.foetus* infection in cats between 0 and 6 months of age was of 18% (764/4327), in cats between 6 months and a year was of 13% (379/2834), from the gap of 1 to 4 years of age the prevalence was the lowest with 10% (402/3927), from 4 to 8-year-old it was shown an incidence of 16% (408/2566) and in cats over the age of 8 the prevalence was of 11% (413/3742).

This information can also be seen in Table 1 below.

From the infected population, 32% were from the group of ages 0-0.5; 16% from the age group 0.5-1: 17% from both 1-4 and 4-8 years old and 18% from the cats <8 years old.

The chi-squared test indicated there to be a significant association between the age od cats and incidence of *T.foetus* (P<0.001).

**Table 1.** Age distribution and prevalence of UK *T.foetus*-positive cats in age groups from samples between February 2010 and October 2017.

|  |  |  |  |
| --- | --- | --- | --- |
| Ages | Total  (n=17396) | Number testing *T.foetus* positive  (n=2357) | Prevalence of *T.foetus (%)* |
| 0-0.5 | 4327 | 764 | 18% |
| 0.5 – 1 | 2834 | 370 | 13% |
| 1 – 4 | 3927 | 402 | 10% |
| 4 – 8 | 2566 | 408 | 16% |
| <8 | 3742 | 413 | 11% |

**Breed**

The prevalence of *T.foetus* in pedigree cats was of 16% (1133/7173), whilst the prevalence of *T.foetus* in non-pedigree (domestic short- or long-hair) cats was 12% (1127/9507). The chi-squared test indicated significantly more pedigree cats to be *T.foetus-*positive compared to non-pedigree cats (P<0.001).

The database contained 35 different breeds (see Appendix I). The statistical analysis was restricted to 18 breed categories containing at least 100 samples displayed in Table 2 below. Domestic Short Hair accounted for the highest infected population with 45%(976/2197) followed by the Bengal breed with 11% m (246/2197). In contrast, the breed in the control population with the lowest number of cats testing *T.foetus-*positive was Tonkinese with only 0.4% (8/2197).

The prevalence of *T.foetus* for each of the 18 breeds can also be found in Table 2.

Abyssinian cats had the highest prevalence in which 24% (28/119) of the cats tested positive, followed by Bengal and Persian in which 22% (246/1134) and 21% (110/519) tested positive, respectively. On the other hand, Tonkinese cats had the lowest prevalence of infection with just 7% (8/111) testing positive.

The chi-squared test indicated there to be a significant association between breed and *T.foetus* infection (P<0.001).

|  |  |  |  |
| --- | --- | --- | --- |
| Breed (n=18) | Total  (n=16214) | Number testing *T.foetus* positive  (n=2197) | Prevalence of *T.foetus* (%) |
| Abyssinian | 119 | 28 | 24 |
| Bengal | 1134 | 246 | 22 |
| Birman | 219 | 44 | 20 |
| British Blue | 172 | 21 | 12 |
| British Shorthair | 669 | 131 | 20 |
| Burmese | 336 | 45 | 13 |
| Domestic Long Hair | 944 | 151 | 16 |
| Domestic Short Hair | 8563 | 976 | 12 |
| Maine Coon | 752 | 75 | 10 |
| Norwegian Forest Cat | 220 | 26 | 12 |
| Oriental | 240 | 32 | 10 |
| Persian | 519 | 110 | 21 |
| Ragdoll | 778 | 119 | 15 |
| Russian Blue | 100 | 20 | 20 |
| Siamese | 935 | 114 | 12 |
| Somali | 106 | 13 | 12 |
| Sphynx | 297 | 38 | 13 |
| Tonkinese | 111 | 8 | 7 |

**Table 2.** Breed distribution and prevalence of UK *T.foetus*-positive cats for breed categories containing 100 samples between February 2010 and October 2017.

**Gender**

The prevalence of *T.foetus* in the female population was 16% (1307/8158), while the prevalence in the male population was off 12% (1149/9575). The chi-squared test indicated to be a significant association between gender and *T.foetus* infection (P<0.001).

Figure 3 shows the distribution of both *T.foetus*-positive and negative samples for gender categories. 

**Figure 3.** Sample distribution for gender categories between February 2010 and October 2017.

**Neutered Status**

The prevalence of *T.foetus* was the highest for female entire cats with 18% (369/2030) of samples testing positive for infection, followed by female neutered cats with 14% (653/4782). The prevalence for male neutered cats was 13% (796/6276), while for male entire the prevalence was the lowest with only 11% (210/6276) of cats testing *T.foetus* positive.

The chi-squared test showed no significant association between male and female neutered status and *T.foetus* infection. (P=0.0618).

When collapsing the variables together entire and neutered cats, the prevalence of *T.foetus* was higher in entire cats with 14% (579/3992) testing positive compared to neutered cats, in which 13% (1449/11058) tested positive.

The chi-squared test indicated no significant association between neutered cats to be *T.foetus*-positive and entire cats.

**Annual Incidence**

Figure 4 shows the incidence of *T.foetus* from 2010 to 2017. The highest incidence occurred in 2017 in which 24% (528/2172) of samples tested positive. Since 2013 the incidence of infection appears to increase every year, with 10% (287/2787) in 2013, 12% (348/2956) in 2014, 14% (8387/2814) in 2015, 18% (511/2172) in 2016 and the peak in 2017 previously mentioned.

Prior to 2013, on the contrast showed low incidence in both 2010 and 2012 with 11% (134/1160); (269/2501) respectively and peak in 2011 reaching 17% (328/1912) of samples testing *T.foetus* positive.

The chi-squared test indicated there to be a significant association between year of sampling and prevalence of *T.foetus* (P<0.01).

**Figure 4.** The UK incidence of *T.foetus* infection 2010 to 2017.

**Season**

The incidence of *T.foetus* was the highest in the Autumn with 29% (802/5571) of samples testing positive. The prevalence was 24% in both Winter and Summer (716/4639) and (595/4633), respectively. In the Spring, the incidence was the lowest at 22% (679/4288).

From the infected population, Autumn accounted for 29% (802/2792), contrasting with Summer with only 21% (595/2797).

The chi-squared test showed a significant association between season and incidence of *T.foetus* (P<0.01).

**Geographical Origin**

The information regarding the prevalence of infection of which district can be seen in Appendix II. The 122 districts were grouped into 13 regions for statistical analysis of which Island of Man hat the highest *T.foetus* prevalence with 33% (6/18) of cats testing positive, whilst South East England had the lowest incidence of infected cats with 12% (413/3383).

Greater London accounted for 21% (559/2724) of the infected population. Contrasting both Channel Islands and Island of Man accounted for less than 1% of the infected population with 0.33% (9/2724) and 0.22% (6/2742), correspondingly.

|  |  |  |  |
| --- | --- | --- | --- |
| Region (n=13) | Total (n=18746) | Number testing *T.foetus* positive (n=2724) | Prevalence of *T.foetus* (%) |
| North West | 1696 | 265 | 16 |
| North East | 1263 | 168 | 13 |
| East England | 1912 | 271 | 14 |
| West Midlands | 1243 | 204 | 16 |
| East Midlands | 1230 | 167 | 14 |
| South West | 1856 | 321 | 17 |
| South East | 3383 | 413 | 12 |
| Greater London | 3939 | 559 | 14 |
| Scotland | 1399 | 205 | 15 |
| Wales | 658 | 114 | 17 |
| Northern Ireland | 90 | 19 | 21 |
| Channel Islands | 59 | 9 | 15 |
| Island of Man | 18 | 6 | 33 |

**Table 3.** Region distribution and prevalence of UK *T.foetus*-positive cats between February 2010 and October 2017.

The chi-squared test indicated there to be a significant association between region and *T.foetus* infection (P<0.001).

**Discussion**

**Prevalence in the UK**

This study revealed that the UK *T.foetus* prevalence in domestic cats between February 2010 and October 2017 to be 14.6% (2797/19111). In agreement with other studies, these results showed a similar prevalence with Gunn-Moore and Tenant (2007) who formerly reported UK prevalence to be 14.4 %, as well as, with Profizi *et al* (2013) that found the prevalence of *T.foetus* infection in France to be 14.3%. However, the overall prevalence in Europen countries is generally lower than in the USA with 31% (Foster *et al.* 2004) and New Zealand with 82% (Kingsbury *et al*. 2010) showing an even higher incidence of infection.

**Determents of Infection**

**Age**

In comparison with similar studies, this study showed a significant association between *T.foetus* and age, in which cats ≤1-year-old were more susceptible to infection (Kuehner *et al* 2011; Profizi *et al*. 2013; Stockdale *et al*. 2009; Xenoulis *et al*.2010). A reasonable explanation for this is that younger cats present an immature immune system and therefore are more susceptible to *T.foetus* infection compared to older cats (Gookin et al. 2009). In the current study, it was found that 48 % (1134/2357) of the infected population was ≤1-year-old, similar results to Profizi *et al.* 2013 with 54% of *T.foetus*-infected cats to be ≤1-year-old.

In contrast, a few studies have found no association between age and *T.foetus* infection such as Tysnes *et al*. (2011), despite the fact, this report involved clinically healthy cats and may have uncovered older asymptomatic carriers as a consequence.

Holiday *et al*. (2009), in opposition to most studies revealed the majority (67%) of *T.foetus*-positive diarrhoetic cats to be over one year of age. Nonetheless, it must be noted this study involved a closed population of cats and for that reason lack of acquired immunity may have permitted older cats to develop clinical signs.

This study had a median age of 8 months among infected cats, similar to former studies from the USA with a median age of 9 months (Gookin et al. 1999; Gookin et al. 2004; Foster et al. 2004) and in France with a median age of 9.5 months (Profizi *et al*. 2013).

Then again, the high incidence of *T.foetus* infection among kittens and young cats may reveal the time point at which transmission is most probable to occur. It is likely, that the risk of faecal-oral transmission of *T.foetus* is utmost between an infected mother and her kittens, and consequently among kitten within the litter (Holiday *et al*. 2009)

**Breed**

Prevalence of *T.foetus* in pedigree breeds was demonstrated to be 16% (1133/7173) whilst the prevalence of *T.foetus* in non-pedigree (domestic short- or long-hair) cats was 12% (1127/9507).

Although it is possible that some pedigree breeds may have genetic factors that predispose them to infection, it is almost certain that is the way in which the cats are managed that predisposed them to a higher chance of infection. This is believed to be that great population density is the most important risk factor for this infection because it predisposed to high levels of faecal-oral spread (Gookin et al. 1999; Foster et al 2004; Gunn-Moore and Tennant. 2007).

It has been implied that faecal samples from pedigree cats are more likely to be submitted, however, this study had approximately equal numbers of pedigree and non-pedigree samples available for analysis suggesting this was not the case in the present study. This is in marked dissimilarity to the general UK data that propose that only 8–12% of domestic pet cats are pedigree ([PFMA 2004](https://www.sciencedirect.com/science/article/pii/S1098612X07000253" \l "bib11), [Tiffin 2006](https://www.sciencedirect.com/science/article/pii/S1098612X07000253" \l "bib12)). That usually indicates that faecal samples from pedigree cats are much more probable to be submitted for *T. foetus* PCR than those from non-pedigree cats. This sampling bias probably arose because breeders and owners of pedigree cats are more expected to be motivated to undertake the investigation. In addition, they may be financially more able to pay for the assay, and through international cat breeding magazines, they may have had an increased awareness of the infection.

This report also indicated there to be a significant association between breed and *T.foetus* infection. The further statistical analysis is required to establish which breed is significantly more affected. The highest prevalence was found in Abyssinian cats in which 24% (28/119) of the cats tested positive. In contrast with other studies that show a high prevalence in Norwegian Forest cats to have the highest prevalences such as Kuenhner *et al*. (2011). However, there's also studies that show no association between breed and T.foetus infection such as Stockdale et al. (2009).

While several breeds such as Bengal and Siamese cats were overrepresented in the present study, only Abyaainianian cats were significantly higher risk of *T.foetus* infection.

There may be various reasons why cats in the UK may have a lower incidence of infection than in the USA, and this could outcome from the lower indoor pedigree cat density that is seen in the UK compared to the USA (Gunn-Moore and Tenant. 2007). It may also vary between different cat breeds as management practices vary and some breeds contain significant numbers of cats imported from the USA.

Domestic Short Hair accounted for the highest infected population with 45% (976/2197), this finding is in accordance with Xenoulis *et al.* 2010 and Profizi *et al.* 2013. However, DSH cats are very commonly kept as pets, while former studies have used cats from catteries and cat shows that are entirely pedigree breeds.

**Gender**

Unlike most studies, this investigation demonstrated a significant association between gender and T.foetus infection (Stockdale et al 2009; Keuhner et al 2011; Profizi et al. 2013).

The prevalence of *T.foetus* in the female population was 16% (1307/8158), while the prevalence in the male population was 12% (1149/9575), these results show an agreement with Gunn-Moore *et al*. (2007) that also showed the gender-ratio to be similar but with a higher incidence in females. Reasons as to why are not yet clear but may have something to do with significantly more female cats being ≤1-year-old (62%) and therefore having undeveloped immune systems. In contrast, Manning (2010) demonstrated male cats to be a great risk factor (P<0.01) for *T.foetus* infection.

**Neutered Status**

Contrasting most studies, there was no significant association between neutered status and *T.foetus* infection. Reasons for this are unclear but percentages of entire vs neutered cats and correlation with the age of cats as well as being pedigree or non-pedigree might’ve influenced the results. The prevalence of *T.foetus* was higher in entire cats with 14% (579/3992) testing positive compared to neutered cats, of which 13% (1449/11058) tested positive. Although, the incidence of infection was higher in entire cats rather than neutered cats, as in agreement with other studies such as Foster *et al.* (2004) Stockdale *et al.* (2009), it showed much lower percentages of positive cases than studies have previously mentioned.

**Annual Incidence**

There was a significant association between year of sampling and *T.foetus* incidence. The highest incidence occurred in 2017 in which 24% (528/2172) of samples tested positive.

Annual incidence was hypotheses to increase, possibly to increase the awareness either via veterinary advice or international breeding magazines (Gunn-Moore *et al*. 2007)

Nonetheless, the study showed an incidence decreased after a peak in 2011. It is unclear as to why such a pattern exists especially when it is possible to rule out several laboratory-based explanations.

Firstly, the PCR technique used remained unchanged throughout the study period including threshold set for *T.foetus* detection. Secondly, a new “feline diarrhoea panel” was obtainable in 2010 presenting several caused of feline diarrhoea with the potential to uncover asymptomatic carriers. This new panel does not explain the high incidence seen in 2011 nor does it explain the decline in incidence subsequently.

**Season**

There was a significant association between season an incidence of *T.foetus.* Prevalence was the highest in the Autumn with 29% (802/5571) of samples testing positive, with incidence of 24% in both Winter and Summer (716/4639) and (595/4633), respectively and in the Spring, the incidence was the lowest at 22% (679/4288), this is in agreement with the knowledge that most gastrointestinal parasitic infection is most prevalent during heavy rainfall and high relative humidity, in the UK that occurs usually in the Autumn and Winter (Soulsby, 1985). However, there is also previous studies that show no seasonal predisposition for *T.foetus* such as Bell *et al.* (2010)

In contrast, with a spatio-temporal study done on *T. foetus* infection in Texas bulls where it was detected a higher prevalence of infection doing Summer, although this was believed to be connected with breeding season, it is yet not clear if exists a seasonal predisposition for *Tririchomonas foetus* (Szonyi *et al*. 2012).

**Geographical Origin**

*Tritrichomonas foetus* has been identified in the domestic cats in many geographic regions. Its geographic distribution has covered four continents including Europe (Austria, Finland, France, Germany, Greece, Italy, Netherland, Norway, Poland, Spain, Sweden, Switzerland, and UK), North America (Canada and USA), Australia/Oceania (Australia and New Zealand), and Asia (Japan and South Korea) (Yao and Koster, 2015). Accordingly, either parasite incidence is currently lower in Europe than in America and Australia or this prevalence is underestimated in European studies.

This investigation showed *T.foetus* to be distributed throughout the UK, with a significant association between region and *T.foetus* infection, The highest prevalence was seen in the Island of Man with 33% (6/18) of cats testing positive, whilst South East England had the lowest incidence of infected cats with 12% (413/3383).

However, these regions had the smallest accounted population, with data collected from cats in catteries, show cats, or presenting clinical signs such as being diarrheic cats, this could be a bias associated with such samples.

Similar to other studies, such as Gunn-Moore and Tenant (2007), Greater London accounted for the greatest amount 21% (559/2724) of the infected population, possibly caused by close proximity of cats in this area. In order to establish which region were significantly more affected requires further statistical analysis beyond the scope of this study.

**Limitation of the study**

It is likely that this investigation has been exposed to sampling bias as positive cats are recognized to shed or deposit parasite in short numbers intermittently, mainly after recent antibiotic use (Gunn-Moore et al. 2007; Holiday et al. 2009). It is, for that reason, likely that certain samples might have fallen below the detection threshold and as a result, were categorized as false negatives.

False positives could have arisen from cross-contamination of samples during spinning and washing of the formalised faecal samples, but specific care was undertaken to avoid that occurrence. In the same way, care was taken to avoid cross-contamination during DNA extraction. Quality controls for PCR were negative, and positive-control size variants displayed no evidence of cross-contamination. Misidentification was ruled out by sequencing two of the representative amplicons. Consequently, it is believed that the results presented here were true positives. Detection of PCR-positive samples could result from the presence of naked DNA isolated from the faecal sample. While this does not negate the fact the sample tested positive for the presence of *T. foetus*, it does not address the state of infection (Kingsbury *et al*. 2010). Thus, some cats may not have had an active *T. foetus* infection but could still have tested positive from residual DNA fragments passed in the faeces, reflecting a recent or failed infection.

A colonic faecal flush might help reduce this, as well as, the veterinarian ensuring that antibiotics are not given at least 7 days prior to faecal collection (Tolbert and Gookin. 2009).

So as to expand this research, further information regarding mother to kitten transmission, potential environmental risk factors including 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, the presence of clinical signs and prevalence in feral cats would have been advantageous to further investigate *T.foetus* transmission. Furthermore, the addition of travel history on laboratory submission forms may encourage a more thorough history allowing better assessment of individuals in multi-cat households as well as *T.foetus* is being reported internationally. Reactions and use of antibiotic to follow *T.foetus*-positive cases to asses the outcome of the disease.

The significance of asymptomatic *T foetus* infection and its role in the epidemiology of the disease are not yet clearly distinct. Previous investigations have mostly focused on the association between intestinal *T foetus* infection and current diarrhoea (Gookin et al. 1999; Gookin et al 2004).

Despite these limitations. This study design allows the most cost-effective way to assess the prevalence and also identify risk factors associated with *T.foetus* infection. The data is still valuable even though there was no information regarding if these cats were healthy or unhealthy, and from multi-cat households or not. Risk factors will still apply regardless of whether the samples came from healthy or unhealthy cats; the only value that is likely to change would be overall prevalence.

**Conclusions**

This study indicates that the prevalence of feline *T.foetus* infection to be less than 15% (14.6%) in the UK and to have increased throughout the years as was hypothesised; however, neutered status did not appear to be a predising factor for infection. Unexpectedly, geographical location and gender were significantly associated with *T.foetus* infection. Age, breed and season predisposition were, as expected, correlated with *T.foetus* infection.

*Tririchomonas foetus* infection seems to be a new and emerging disease, as well as an important, common and previously unrecognised cause of diarrhoea in cats in this country. Therefore, it is an important differential diagnosis to consider when investigating cats showing clinical signs, especially in animals ≤1-year-old.

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**Appendix I**

Breed distribution and prevalence of UK *T.foetus*-positive cats for breed categories (n=35) between February 2010 and October 2017.

|  |  |  |  |
| --- | --- | --- | --- |
| Breed  (n=35) | Total  (n=16680) | Number testing *T.foetus* positive  (n=2260) | Prevalence of *T.foetus* (%) |
| Abyssinian | 119 | 28 | 24 |
| American Short Hair | 6 | 2 | 33 |
| Balinese | 25 | 3 | 12 |
| Bengal | 1134 | 246 | 22 |
| Birman | 219 | 44 | 20 |
| British Blue | 172 | 21 | 12 |
| British Shorthair | 669 | 131 | 20 |
| Burmese | 336 | 45 | 13 |
| Burmilla | 47 | 8 | 17 |
| Cornish Rex | 21 | 1 | 5 |
| Domestic Long Hair | 944 | 151 | 16 |
| Devon Rex | 93 | 9 | 10 |
| Domestic Short Hair | 8563 | 976 | 12 |
| Egyptian Mau | 33 | 6 | 18 |
| Havana Brown | 27 | 3 | 11 |
| Korat | 23 | 2 | 9 |
| Maine Coon | 752 | 75 | 10 |
| Munchkin | 2 | 0 | 0 |
| Norwegian Forest Cat | 220 | 26 | 12 |
| Ocicat | 11 | 1 | 9 |
| Oriental | 240 | 32 | 10 |
| Persian | 519 | 110 | 21 |
| Pixie-Bon | 15 | 4 | 27 |
| Ragamuffin | 27 | 4 | 15 |
| Ragdoll | 778 | 119 | 15 |
| Russian Blue | 100 | 20 | 20 |
| Scottish Fold | 38 | 9 | 24 |
| Selkirk Rex | 25 | 5 | 20 |
| Siamese | 935 | 114 | 12 |
| Somali | 106 | 13 | 12 |
| Sphynx | 297 | 38 | 13 |
| Tiffany | 66 | 6 | 9 |
| Tonkinese | 111 | 8 | 7 |
| Turkish Angora | 2 | 0 | 0 |
| Turkish Van | 5 | 0 | 0 |

**Appendix II**

District distribution and prevalence of UK *T.foetus*-positive cats between February 2010 and October 2017.

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