



UNIVERSITI PUTRA MALAYSIA

***DETECTION OF GASTROINTESTINAL PROTOZOA IN PET CATS
PRESENTED TO SELECTED VETERINARY CLINICS IN THE
KLANG VALLEY AND RISK FACTORS ASSOCIATED WITH
INFECTION***

TAN LI PING

FPV 2016 60

**DETECTION OF GASTROINTESTINAL PROTOZOA IN PET CATS
PRESENTED TO SELECTED VETERINARY CLINICS IN THE KLANG
VALLEY AND RISK FACTORS ASSOCIATED WITH INFECTION**

TAN LI PING

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan

MARCH 2016

CERTIFICATION

It is hereby certified that I have read this paper entitled, "Detection of gastrointestinal protozoa in pet cats presented to selected veterinary clinics in the Klang Valley and risk factors associated with infection" by Tan Li Ping, and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 –Project.

It is hereby certified that we have read this project paper entitled "Detection of gastrointestinal protozoa in pet cats presented to selected veterinary clinics in the Klang Valley and risk factors associated with infection" by Tan Li Ping and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 - Project.

ASSOC. PROF. DR. MALAIKA WATANABE

DVM (UPM), PhD (Yamaguchi University)

Associate Professor,

Department of Companion Animal Medicine and Surgery,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

DR. REUBEN SUNIL KUMAR SHARMA

DVM (UPM), MSc (UPM), PhD (University of Cambridge)

Lecturer,

Department of Veterinary Laboratory Diagnostics

Faculty of Veterinary Medicine

University Putra Malaysia

(Co-Supervisor)

DR. PUTERI AZAZIAH MEGAT ABDUL RANI

DVM (UPM), PhD (University of Queensland)

Lecturer,

Department of Companion Animal Medicine and Surgery

Faculty of Veterinary Medicine

University Putra Malaysia

(Co-Supervisor)

ACKNOWLEDGMENT

First and foremost, I would like to express my highest gratitude to my supervisor Associate Professor Dr. Malaika Watanabe for all her patience, guidance, advice and support throughout the project.

Also I would like to thank both of my co-supervisors Dr. Reuben Sunil Kumar Sharma and Dr. Puteri Azaziah Megat Abdul Rani for their help and guidance in the past few months.

The University Veterinary Hospital veterinary officers and supporting staff, Parasitology lab staff as well as the Bacteriology lab staff and students. They have been a huge support towards the completion of this project. I am deeply grateful for all their guidance, knowledge and help.

Not forgetting the veterinarians and staff of St. Angel Animal Medical Centre and Hospital Veterinar Kuala Lumpur that helped me during sample collection for my project.

A special thanks to Prof Ariff, Dr Latifah, Puan Maizatul, En. Rashid, Puan Fauziah, Ruvi, Dr Vish for all their patience and knowledge on the laboratory techniques.

Last but not least, I would like to thank my family for their moral support and encouragement throughout the project. Without all of the people mentioned above, this project would not have been possible. Thank you.

CONTENTS

	Page
TITLE	i
CERTIFICATION	ii
ACKNOWLEDGEMENTS	v
CONTENTS	vi
ABSTRACT	viii
ABSTRAK	x
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	4
2.1 Common gastrointestinal protozoa in cats	4
2.2 Prevalence and epidemiology of gastrointestinal protozoa in cats	7
2.3 Diagnostic methods for gastrointestinal protozoa in cats . .	10
3.0 MATERIALS AND METHODS	16
3.1 Sample population	16
3.2 Sample collection	16
3.3 Sample processing	16
3.4 Interpretation of results	17
3.5 Data analysis	18
4.0 RESULTS	19

4.1	Study population	19
4.2	<i>Tritrichomonas foetus</i> culture	20
4.3	Overall prevalence of gastrointestinal protozoal infections in pet cats	21
5.0	DISCUSSION	27
6.0	CONCLUSION	32
7.0	RECOMMENDATIONS	32
	REFERENCES	33
	APPENDICES	48

ABSTRACT

**DETECTION OF GASTROINTESTINAL PROTOZOA IN PET CATS
PRESENTED TO SELECTED VETERINARY CLINICS IN THE KLANG
VALLEY AND RISK FACTORS ASSOCIATED WITH INFECTION**

By

Tan Li Ping

2016

Supervisor: Assoc. Prof. Dr. Malaika Watanabe

Co-supervisor: Dr. Reuben Sunil Kumar Sharma

Dr. Puteri Azaziah Megat Abdul Rani

The common gastrointestinal protozoa in cats that cause diarrhea are *Giardia* spp., *Isospora* spp. and *Cryptosporidium* spp., and recently *Tritrichomonas foetus* has been recognized as an emerging protozoa that causes chronic diarrhea in cats. *Tritrichomonas foetus* infection in cats has not yet been reported in Malaysia.

Entamoeba spp. is found rarely but present in cats. This study aimed to investigate the prevalence of gastrointestinal protozoa in pet cats presented to selected veterinary clinics in Klang Valley as well as the risk factors associated with these protozoal infections. Rectal swabs were performed on 30 diarrheic cats presented to selected veterinary clinics in the Klang Valley to culture *Tritrichomonas foetus*. Another 30 fecal samples were collected randomly and subjected to staining for the detection of other gastrointestinal protozoa. Two out of 30 culture samples were positive for *Tritrichomonas foetus* with a prevalence of 6.7% and both positive samples were from young kittens. *Cryptosporidium spp.* was the only protozoa detected in 3 out of 30 samples through the staining method with a prevalence of 10%. This study detected *Tritrichomonas foetus* for the first time in the Malaysian cat population. The overall prevalence of gastrointestinal protozoa in pet cats in the Klang Valley was low.

Keywords : Gastrointestinal protozoa, *Tritrichomonas foetus*, Cat, Culture, Staining

ABSTRAK

**PENGESANAN PROTOZOA GASTRUSUS DALAM KUCING
PERLIHARAAN DIBAWA KE KLINK VETERINAR TERPILIH DI KLANG
VALLEY DAN FAKTOR-FAKTOR RISIKO BERKAITAN DENGAN INFEKSI.**

Oleh

Tan Li Ping

2016

Penyelia: Assoc. Prof. Dr. Malaika Watanabe

Penyelia Bersama: Dr. Reuben Sunil Kumar Sharma

Dr. Puteri Azaziah Megat Abdul Rani

Protozoa gastrousus sepunya dalam kucing yang menyebabkan cirit-birit termasuk *Giardiaspp.*, *Isospora spp.* and *Cryptosporidium spp.*, dan kebelakangan ini *Tritrichomonas foetus* telah dikenali sebagai protozoa yang menyebabkan cirit-birit kronik dalam kucing. Infeksi *Tritrichomonas foetus* dalam kucing belum dilaporkan di

Malaysia. *Entamoeba spp.* jarang ditemui tetapi kadang-kadang muncul dalam kucing. Kajian ini bertujuan untuk menyiasat prevalens protozoa gastrousus dalam kucing perliharaan dibawa klinik veterinar terpilih dan juga faktor-faktor risiko berkaitan dengan infeksi protozoa. Pengesatan rektum dilakukan pada 30 kucing cirit-birit yang dibawa ke klinik veterinar terpilih di Klang Valley untuk kultur *Tritrichomonas foetus*. 30 sampel najis dikumpul secara rambang dan diwarnakan dengan teknik pewarnaan untuk mengesan protozoa gastrousus lain. Dua daripada 30 sampel kultur positif untuk *Tritrichomonas foetus* dengan prevalens 6.7% dan dua sampel positif tersebut adalah daripada anak kucing. *Cryptosporidium spp.* adalah satu-satu protozoa yang ditemui dalam 3 daripada 30 sampel dengan penggunaan teknik pewarnaan dengan prevalens 10%. Kajian ini telah mengesan *Tritrichomonas foetus* untuk kali pertama di populasi kucing di Malaysia. Prevalens keseluruhan untuk protozoa gastrousus di kucing perliharaan di Klang Valley adalah rendah.

Kata kunci : protozoa gastrousus, *Tritrichomonas foetus*, kucing, kultur, pewarnaan



1.0 INTRODUCTION

Protozoans are unicellular organisms that belong to the kingdom Protista which is further divided into several phyla(Hendrix and Robinson, 2006). The four different phyla of veterinary importance includes the flagellated protozoans, amoeboid protozoans, apicomplexans and ciliated protozoans (Hendrix and Robinson, 2006). Protozoa that infect domestic animals may reside in the gastrointestinal tract, circulatory system, urogenital system and also in the respiratory system (Hendrix and Robinson, 2006). Every phyla of unicellular protozoans have their own lifecycle, reproduction stage as well as their feeding behavior. They are transmitted from one host species to another via different means. Generally, protozoans are transmitted via four different routes, including direct contact, exposure to resistant stages in the environment, ingestion and through vectors such as blood feeding arthropods (Bowman et al, 2002).

Some gastrointestinal protozoa are known to cause chronic diarrhea in the feline species. The gastrointestinal protozoa of pathogenic importance in cats include *Giardia spp.*, *Tritrichomonas foetus* and *Cryptosporidium spp.* which cause problems and shed more in younger cats or kittens (Zajac and Conboy, 2012 ; Gookin et al, 2001 ; Craven, 2010; Rambozzi, 2007). *Tritrichomans foetus* has recently garnered attention as an important aetiological agent of chronic diarrhea in cats in the United States and Europe. *Isospora spp.* however usually causes infections without obvious clinical signs, even though occasional mild diarrhea might be observed in very young and

immunosuppressed cat (Craven, 2010). *Toxoplasma gondii* is a common coccidian protozoa in cats that may or may not cause clinical signs (DeFeo et al, 2002).

The host of these protozoa, cats, are primarily kept as companion animals. As more and more people keep these domesticated cats as pets which share the same household as humans, the frequency of contact between them increases. This leads to an increased risk of zoonotic disease transmission between the feline species and humans. These gastrointestinal protozoa are not just clinically important for cats but also carry a zoonotic risk to humans and subsequently pose a one health problem.

Despite the large population of stray and pet cats in Malaysia, not many studies have been carried out in Malaysia on gastrointestinal protozoa of cats. The most recent study was done by Ngui et al, 2014 in Malaysia on the gastrointestinal protozoa of cats in the stray population. Furthermore, there has been a few studies on the detection and prevalence of *Tritrichomonas foetus* in cats in Malaysia. A lot of studies have shown that the prevalence of gastrointestinal protozoa is higher in younger animals, diarrheic cats as well as cats kept in multi-cat households (Barutzki and Schaper, 2013 ; Labarthe, et al, 2008 ; Zajac and Conboy, 2012 ; Rambozzi, 2007). The paucity of available and current data on the gastrointestinal protozoa of pet cats in Malaysia and the lack of any studies on *Tritrichomonas foetus* in cats in Malaysia warranted this study.

The objectives of this study were to:-

1. detect the gastrointestinal protozoa in pet cats presented to selected veterinary clinics in Klang Valley.
2. determine the prevalence of gastrointestinal protozoa in pet cats presented to selected veterinary clinics in Klang Valley.
3. investigate risk factors associated with infection with these protozoa.

Performing Polymerase Chain Reaction (PCR) to detect gastrointestinal protozoa due to its higher sensitivity and the ability to detect up to the species level.

REFERENCES

- Agrawal, N., Sharma, U., & Sharma, A. K. (2006). Trichrome staining for detection of intestinal protozoa a better screening method. *The Journal of communicable diseases*, 38(4), 351-354.
- Ålapeta, J., Craig, S., McDonell, D., & Emery, D. (2010). *Tritrichomonas foetus* from domestic cats and cattle are genetically distinct. *Experimental Parasitology*, 126(2), 209-213. Retrieved 28th August 2015 from <http://dx.doi.org/10.1016/j.exppara.2010.04.024>
- Al-Aredhi, H. (2015). Prevalence of gastrointestinal parasites in domestic cats(*Felis catus*) in Al-Diwaniya province/Iraq. *International Journal Of Current Microbiology And Applied Sciences*, 4(5), 166-171. Retrieved 13th January 2016 from <http://www.ijcmas.com>
- Ballweber, L., Panuska, C., Huston, C., Vasilopoulos, R., Pharr, G., & Mackin, A. (2009). Prevalence of and risk factors associated with shedding of Cryptosporidium felis in domestic cats of Mississippi and Alabama. *Veterinary Parasitology*, 160(3-4), 306-310. Retrieved 28th August 2015 from <http://dx.doi.org/10.1016/j.vetpar.2008.11.018>
- Barutzki, D., & Schaper, R. (2011). Results of Parasitological Examinations of Faecal Samples from Cats and Dogs in Germany between 2003 and 2010. *Parasitology Research*, 109(S1), 45-60. Retrieved 3rd of January 2016 from <http://dx.doi.org/10.1007/s00436-011-2402-8>

- Bissett, S., Stone, M., Malik, R., Norris, J., O'Brien, C., & Mansfield, C. et al. (2009). Observed occurrence of *Tritrichomonas foetus* and other enteric parasites in Australian cattery and shelter cats. *Journal Of Feline Medicine & Surgery*, 11(10), 803-807. Retrieved 9th September 2015 from <http://dx.doi.org/10.1016/j.jfms.2009.02.001>
- Bowman, D., & Lucio-Forster, A. (2010). Cryptosporidiosis and giardiasis in dogs and cats: Veterinary and public health importance. *Experimental Parasitology*, 124(1), 121-127. Retrieved 12th December 2015 from <http://dx.doi.org/10.1016/j.exppara.2009.01.003>
- Bowman, D., Lynn, R., Eberhard, M., & Alcaraz, a. (2003). *Georgis' Parasitology for Veterinarians*(8th ed., pp. 83-96, 317-319). Missouri: Saunders.
- Brook, E., Christley, R., French, N., & Hart, C. (2007). Detection of Cryptosporidium oocysts in fresh and frozen cattle faeces: comparison of three methods. *Letters In Applied Microbiology*, 0(0), Retrieved 8th January 2016 from <http://dx.doi.org/10.1111/j.1472-765x.2007.02257.x>
- Ceplecha, V., Svoboda, M., Čepička, I., Husník, R., Horáčková, K., & Svobodová, V. (2013). InPouch™ TF-Feline medium is not specific for *Tritrichomonas foetus*. *Veterinary Parasitology*, 196(3-4), 503-505. <http://dx.doi.org/10.1016/j.vetpar.2013.04.015>
- Chomel, B., & Sun, B. (2011). Zoonoses in the Bedroom. *Emerg. Infect. Dis.*, 17(2), 167-172. Retrieved 10th January 2016 from <http://dx.doi.org/10.3201/eid1702.101070>
- Craven, M. (2010). Management of Kitten Diarrhea. *NAVC Clinician's Brief*, 59-63.
- Cryptosporidium spp. and other zoonotic enteric parasites in a sample of

domestic dogs and cats in Niagara region of Ontario. (2006). *Canadian Veterinary Journal*, 47, 1179-1184.

Dabritz, H. A., Miller, M. A., Atwill, E. R., Gardner, I. A., Leutenegger, C. M., Melli, A. C., & Conrad, P. A. (2007). Detection of Toxoplasma gondii-like oocysts in cat feces and estimates of the environmental oocyst burden. *Journal of the American Veterinary Medical Association*, 231(11), 1676-1684.

Dahlgren, S., Gjerde, B., & Pettersen, H. (2007). First record of natural *Tritrichomonas foetus* infection of the feline uterus. *J Small Animal Practice*, 48(11), 654-657. Retrieved 10th October 2015 from <http://dx.doi.org/10.1111/j.17485827.2007.00405.x>

Day, M., Breitschwerdt, E., Cleaveland, S., Karkare, U., Khanna, C., & Kirpensteijn, J. et al. (2012). Surveillance of Zoonotic Infectious Disease Transmitted by Small Companion Animals. *Emerg. Infect. Dis.*, 18(12). Retrieved 12th January 2016 from <http://dx.doi.org/10.3201/eid1812.120664>

DeFeo, M., Dubey, J., Mather, T., & Rhodes III, R. (2002). Epidemiologic investigation of seroprevalence of antibodies to Toxoplasma gondii in cats and rodents. *American Journal of Veterinary Research*, 63(12), 1714-1717. <http://dx.doi.org/10.2460/ajvr.2002.63.1714>

Doi, J., Hirota, J., Morita, A., Fukushima, K., Kamijyo, H., Ohta, H.. & Oku, Y. (2012). Intestinal *Tritrichomonas suis* (= *T. foetus*) infection in Japanese cats. *Journal of Veterinary Medical Science*, 74(4), 413-417.

Dryden, M., & Payne, P. (2010). Fecal Examination Techniques. *NAVC Clinician's Brief*, (April 2010), 13-16.

Foster, D., Gookin, J., Poore, M., Stebbins, M., & Levy, M. (2004). Outcome of

- cats with diarrhea and *Tritrichomonas foetus* infection. *Journal Of The American Veterinary Medicine Association*, 225(6), 888-892.
- Fotedar, R., Stark, D., Beebe, N., Marriott, D., Ellis, J., & Harkness, J. (2007). Laboratory Diagnostic Techniques for Entamoeba Species. *Clinical Microbiology Reviews*, 20(3), 511-532. Retrieved 3rd September 2015 from <http://dx.doi.org/10.1128/cmr.00004-07>
- Frey, C., Schild, M., Hemphill, A., StÃ¼nzi, P., MÃ¼ller, N., Gottstein, B., & Burgener, I. (2008). Intestinal *Tritrichomonas foetus* infection in cats in Switzerland detected by in vitro cultivation and PCR. *Parasitology Research*, 104(4), 783-788. Retrieved 10th January 2016 from <http://dx.doi.org/10.1007/s00436-008-1255-2>
- Gookin, J., Birkenheuer, A., Breitschwerdt, E., & Levy, M. (2002). Single-Tube Nested PCR for Detection of *Tritrichomonas foetus* in Feline Feces. *Journal Of Clinical Microbiology*, 40(11), 4126-4130. Retrieved 11th October 2015 from <http://dx.doi.org/10.1128/jcm.40.11.4126-4130.2002>
- Gookin, J., Foster, D., Poore, M., Stebbins, M., & Levy, M. (2003). Use of a commercially available culture system for diagnosis of *Tritrichomonas foetus* infection in cats. *Journal Of The America Veterinary Medicine Association*, 222(10), 1376-1379.
- Gookin, J., Levy, M., Law, J., Papich, M., Poore, M., & Breitschwerdt, E. (2001). Experimental Infection of cats with *Tritrichomonas foetus*. *American Journal Of Veterinary Research*, 62(11), 1690-1697.
- Gookin, J., Stauffer, S., & Levy, M. (2007). Identification of *Pentatrichomonas hominis* in feline fecal samples by polymerase chain reaction assay. *Veterinary Parasitology*, 145(1-2), 11-15. Retrieved 11th October 2015 from <http://dx.doi.org/10.1016/j.vetpar.2006.10.020>

- Gookin, J., Stebbins, M., Hunt, E., Burlone, K., Fulton, M., & Hochel, R. et al. (2003). Prevalence of and Risk Factors for Feline *Tritrichomonas foetus* and Giardia Infection. *Journal of Clinical Microbiology*, 42(6), 2707-2710.
- Gow, A., Gow, D., Hall, E., Langton, D., Clarke, C., & Papasouliotis, K. (2009). Prevalence of potentially pathogenic enteric organisms in clinically healthy kittens in the UK. *Journal of Feline Medicine & Surgery*, 11(8), 655-662. <http://dx.doi.org/10.1016/j.jfms.2008.12.007>
- Gracenea, M., Gómez, M., & Torres, J. (2009). Prevalence of intestinal parasites in shelter dogs and cats in the metropolitan area of Barcelona (Spain). *Acta Parasitologica*, 54(1). <http://dx.doi.org/10.2478/s11686-009-0005-7>
- Gunn-Moore, D., McCann, T., Reed, N., Simpson, K., & Tennant, B. (2007). Prevalence of *Tritrichomonas foetus* infection in cats with diarrhoea in the UK. *Journal of Feline Medicine & Surgery*, 9(3), 214-218. <http://dx.doi.org/10.1016/j.jfms.2007.01.003>
- Hale, S., Norris, J. M., & Šlapeta, J. (2009). Prolonged resilience of *Tritrichomonas foetus* in cat faeces at ambient temperature. *Veterinary parasitology*, 166(1), 60-65.
- Hartmann, J., Hu, K., He, C. Y., Pelletier, L., Roos, D. S., & Warren, G. (2006). Golgi and centrosome cycles in Toxoplasma gondii. *Molecular and biochemical parasitology*, 145(1), 125-127.
- Hendrix, C., Robinson, E., & Hendrix, C. (2006). *Diagnostic parasitology for veterinary technicians*. St. Louis, Mo.: Elsevier Mosby.
- Hill, S., Cheney, J., Taton-Allen, G., Reif, J., Bruns, C., & Lappin, M. (2000). Prevalence of enteric zoonotic organisms in cats. *Journal of American Veterinary Medical Association*, 257(11), 1441-1446.

Veterinary Medicine Association, 216(5), 687-692.

Holliday, M., Deni, D., & Gunnmoore, D. (2009). *Tritrichomonas foetus* infection in cats with diarrhoea in a rescue colony in Italy. *Journal of Feline Medicine & Surgery, 11*(2), 131-134. Retrieved 11th October 2015 from <http://dx.doi.org/10.1016/j.jfms.2008.06.004>

- Hoopes, J., Polley, L., Wagner, B., & Jenkins, E. (2013). Retrospective investigation of feline gastrointestinal parasites in Western Canada. *Canadian Veterinary Journal, 54*(April).
- Ignatius, R., Eisenblätter, M., Regnath, T., Mansmann, U., Futh, U., Hahn, H., & Wagner, J. (1997). Efficacy of different methods for detection of low *Cryptosporidium parvum* oocyst numbers or antigen concentrations in stool specimens. *European Journal of Clinical Microbiology & Infectious Diseases, 16*(10), 732-736. Retrieved 28th December 2015 from <http://dx.doi.org/10.1007/bf01709253>
- Kehl, K., Cicirello, H., & Havens, P. (1995). Comparison of Four Different Methods for Detection of Cryptosporidium Species. *Journal of Clinical Microbiology, Feb, 1995*, 416-418.
- Kellogg, J., & Elder, C. (1999). Justification for Use of a Single Trichrome Stain as the Sole Means for Routine Detection of Intestinal Parasites in Concentrated Stool Specimens. *Journal of Clinical Microbiology, 37*(3).
- Khademvatan, S., Abdizadeh, R., Rahim, F., Hashemitabar, M., & Tavalla, M. (2014). Stray Cats Gastrointestinal Parasites and its Association With Public Health in Ahvaz City, South Western of Iran. *Jundishapur J Microbiol, 7*(7). <http://dx.doi.org/10.5812/jjm.11079>
- Khan, A., Mergani, M., Mohammed, M., Bano, M., & Khan, N. (2014). Detection of intestinal protozoa by using different methods. *Dent Med Res, 2*(2), 28. <http://dx.doi.org/10.4103/2348-1471.143326>
- Kingsbury, D., Marks, S., Cave, N., & Grahn, R. (2010). Identification of *Tritrichomonas foetus* and Giardia spp. infection in pedigree show cats in New Zealand. *New Zealand Veterinary Journal, 58*(1), 6-10. Retrieved 13th January 2016 from <http://dx.doi.org/10.1080/00480169.2010.65054>

- Koontz, F., & Weinstock, J. (1996). THE APPROACH TO STOOL EXAMINATION FOR PARASITES. *Gastroenterology Clinics of North America*, 25(3), 435-449. [http://dx.doi.org/10.1016/s0889-8553\(05\)70257-0](http://dx.doi.org/10.1016/s0889-8553(05)70257-0)
- Kuehner, K., Marks, S., Kass, P., Sauter-Louis, C., Grahn, R., Barutzki, D., & Hartmann, K. (2011). *Tritrichomonas foetus* infection in purebred cats in Germany: Prevalence of clinical signs and the role of co-infection with other enteroparasites. *Journal of Feline Medicine & Surgery*, 13(4), 251-258. Retrieved 13th January 2016 from <http://dx.doi.org/10.1016/j.jfms.2010.12.002>
- Kuzehkanan, A., Rezaeian, M., Zeraati, H., Mohebali, M., Meamar, A., & Babaei, Z. et al. (2011). A Sensitive and Specific PCR Based Method for Identification of *Cryptosporidium Sp.* Using New Primers from 18S Ribosomal RNA. *Iranian J Parasitol*, 6(4), 1-7. Retrieved 25th January 2016 from <http://ijpa.tums.ac.ir>
- Labarthe, N., Serrão, M., Ferreira, A., Almeida, N., & Guerrero, J. (2004). A survey of gastrointestinal helminths in cats of the metropolitan region of Rio de Janeiro, Brazil. *Veterinary Parasitology*, 123(1-2), 133-139. <http://dx.doi.org/10.1016/j.vetpar.2004.06.002>
- Laude, A., Valot, S., Desoubeaux, G., Argy, N., Nourrisson, C., & Pomares, C. et al. (2015). Is real-time PCR-based diagnosis similar in performance to routine parasitological examination for the identification of *Giardia intestinalis*, *Cryptosporidium parvum/Cryptosporidium hominis* and *Entamoeba histolytica* from stool samples? Evaluation of a new commercial multiplex PCR assay and literature review. *Clinical Microbiology And Infection*. Retrieved 26th January 2016 from <http://dx.doi.org/10.1016/j.cmi.2015.10.019>

- Lee, S., Kim, N., Chae, H., Cho, S., Nam, H., & Lee, W. et al. (2011). Prevalence of *Toxoplasma gondii* Infection in Feral Cats in Seoul, Korea. *Journal of Parasitology*, 97(1), 153-155. Retrieved 1st January 2016 from <http://dx.doi.org/10.1645/ge-2455.1>
- Levy, J., Lappin, M., Glaser, A., Birkenheuer, A., Anderson, T., & Edinboro, C. (2011). Prevalence of infectious diseases in cats and dogs rescued following Hurricane Katrina. *Journal of The American Veterinary Medical Association*, 238(3), 311-317. Retrieved 21th November 2015 from <http://dx.doi.org/10.2460/javma.238.3.311>
- Levy, M., Gookin, J., Poore, M., Birkenheuer, A., Dykstra, M., & Litaker, R. (2003). *Tritrichomonas Foetus* and not *Pentatrichomonas hominis* is the Agent of Feline Trichomonial Diarrhea. *American Society of Parasitologica*, 89(1), 99-104. Retrieved 11th October 2015 from <http://www.bioone.org/doi/full/10.1645/0022-3395%282003%29089%5B0099%3ATFANPH %5D2.0.CO%3B2>
- Lim, S., Park, S., Ahn, K., Oh, D., Ryu, J., & Shin, S. (2010). First Report of Feline Intestinal Trichomoniasis Caused by *Tritrichomonas foetus* in Korea. *Korean J Parasitol*, 48(3), 247. Retrieved 13th September 2015 from <http://dx.doi.org/10.3347/kjp.2010.48.3.247>
- Lindsay, D., & Zajac, A. (2004). Cryptosporidium Infections in Cats and Dogs. *Virginia-Maryland Regional College of Veterinary Medicine*, 864-874.
- Lucio-Forster, A., Griffiths, J., Cama, V., Xiao, L., & Bowman, D. (2010). Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. *Trends In Parasitology*, 26(4), 174-179. Retrieved 24th October 2015 from <http://dx.doi.org/10.1016/j.pt.2010.01.004>

- Mancianti, F., Nardoni, S., Mugnaini, L., Zambernardi, L., Guerrini, A., Gazzola, V., & Papini, R. (2014). A retrospective molecular study of select intestinal protozoa in healthy pet cats from Italy. *Journal Of Feline Medicine And Surgery*, 17(2), 163-167. Retrieved 16th August 2015 from <http://dx.doi.org/10.1177/1098612x14533549>
- Manning, K. (2010). Update on the Diagnosis and Management of *Tritrichomonas foetus* Infections in Cats. *Topics In Companion Animal Medicine*, 25(3), 145-148. Retrieved 7th of September 2015 from <http://dx.doi.org/10.1053/j.tcam.2010.08.001>
- Mardell, E., & Sparkes, A. (2006). Chronic diarrhoea associated with *Tritrichomonas foetus* infection in a British cat. *Veterinary Record*, 158(22), 765-766. Retrieved 7th of September 20145 from <http://dx.doi.org/10.1136/vr.158.22.765>
- McGlade, T., Robertson, I., Elliot, A., & Thompson, R. (2003). High Prevalence of Giardia detected in cats by PCR. *Veterinary Parasitology*, 110(3-4), 197-205. [http://dx.doi.org/10.1016/s0304-4017\(02\)00322-9](http://dx.doi.org/10.1016/s0304-4017(02)00322-9)
- Mekaru, S., Marks, S., Felley, A., Chouicha, N., & Kass, P. (2007). Comparison of Direct Immunofluorescence, Immunoassays, and Fecal Flotation for Detection of *Cryptosporidium spp.* and *Giardia spp.* in Naturally Exposed Cats in 4 Northern California Animal Shelters. *Journal Of Veterinary Internal Medicine*, 21(5), 959.
- Miro, G., Hernandez, L., Montoya, A., Arranz-Solis, D., Dado, D., & Rojo-Montejo, S. et al. (2011). First description of naturally acquired *Tritrichomonas foetus* infection in a Persian cattery in Spain. *Parasitology Research*, 109(4), 1151-1154. Retrieved 7th of September from <http://dx.doi.org/10.1007/s00436-011-2359-7>

- Mostegl, M., Wetscher, A., Richter, B., Nedorost, N., Dinhopl, N., & Weissenback H. (2012). Detection of *Tritrichomonas foetus* and *Pentatrichomonas hominis* in intestinal tissue specimens of cats by chromogenic in situ hybridization. *Veterinary Parasitology*, 183(3-4), 209-214. Retrieved 7th September 2015 from <http://dx.doi.org/10.1016/j.vetpar.2011.07.050>
- Ng, J., Eastwood, K., Durrheim, D., Massey, P., Walker, B., Armson, A., & Ryan, U. (2008). Evidence supporting zoonotic transmission of Cryptosporidium in rural New South Wales. *Experimental Parasitology*, 119(1), 192-195. Retrieved 11th October 2015 from <http://dx.doi.org/10.1016/j.exppara.2008.01.010>
- Ngui, R., Lee, S., Yap, N., Tan, T., Aidil, R., & Chua, K. et al. (2014). Gastrointestinal parasites in rural dogs and cats in Selangor and Pahang states in Peninsular Malaysia. *Acta Parasitologica*, 59(4). Retrieved 28th August 2015 from <http://dx.doi.org/10.2478/s11686-014-0306-3>
- Olson, M., Leonard, N., & Strout, J. (2010). Prevalence and diagnosis of Giardia infection in dogs and cats using a fecal antigen test and fecal smear. *Canadian Veterinary Journal*, 51, 640-642.
- Overgaauw, P., van Zutphen, L., Hoek, D., Yaya, F., Roelfsema, J., & Pinelli, E. et al. (2009). Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. *Veterinary Parasitology*, 163(1-2), 115-122. Retrieved 28th August 2015 from <http://dx.doi.org/10.1016/j.vetpar.2009.03.044>
- Pacheco, F. T., Silva, R. K., Martins, A. S., Oliveira, R. R., Alcântara-Neves, N. M., Silva, M. P., ... & Teixeira, M. C. (2013). Differences in the detection of Cryptosporidium and Isospora (Cystoisospora) oocysts according to the fecal concentration or staining method used in a clinical laboratory. *The*

Journal of parasitology, 99(6), 1002-1008.

Pallant, L., Barutzki, D., Schaper, R., & Thompson, R. (2015). The epidemiology of infections with Giardia species and genotypes in well cared for dogs and cats in Germany. *Parasites & Vectors, 8(1), 2.* Retrieved 11th January 2016 from <http://dx.doi.org/10.1186/s13071-014-0615-2>

Palmer, C., Thompson, R., Traub, R., Rees, R., & Robertson, I. (2008). National study of the gastrointestinal parasites of dogs and cats in Australia. *Veterinary Parasitology, 151(2-4), 181-190.* Retrieved 9th September 2015 from <http://dx.doi.org/10.1016/j.vetpar.2007.10.015>

Palmer, C., Traub, R., Robertson, I., Devlin, G., Rees, R., & Thompson, R. (2008). Determining the zoonotic significance of Giardia and Cryptosporidium in Australian dogs and cats. *Veterinary Parasitology, 154(1-2), 142-147.* Retrieve 11th October 2015 from <http://dx.doi.org/10.1016/j.vetpar.2008.02.031>

Paoletti, B., Otranto, D., Weigl, S., Giangaspero, A., Cesare, A., & Traversa, D. (2011). Prevalence and genetic characterization of Giardia and Cryptosporidium in cats from Italy. *Research In Veterinary Science, 91(3), 397-399.* Retrieved 7th September from <http://dx.doi.org/10.1016/j.rvsc.2010.09.011>

Payne, P., & Artzer, M. (2009). The Biology and Control of *Giardia spp* and *Tritrichomonas foetus*. *Veterinary Clinics of North America: Small Animal Practice, 39(6), 993-1007.* Retrieved 7th September 2015 from <http://dx.doi.org/10.1016/j.cvsm.2009.06.007>

- Rambozzi, L., Menzano, A., Mannelli, A., Romano, S., & Isaia, M. (2007). Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors. *Journal of Feline Medicine & Surgery*, 9(5), 392-396. Retrieved 7th September 2015 from <http://dx.doi.org/10.1016/j.jfms.2007.03.005>
- Reinmann, K., MÃ¼ller, N., Kuhnert, P., Campero, C., Leitsch, D., & Hess, M. et al. (2012). *Tritrichomonas foetus* isolates from cats and cattle show minor genetic differences in unrelated loci ITS-2 and EF-1 \pm . *Veterinary Parasitology*, 185(2-4), 138-144. Retrieved 28th August 2015 from <http://dx.doi.org/10.1016/j.vetpar.2011.09.032>
- Rigo, C. R., & Franco, R. M. B. (2002). Comparison between the modified Ziehl-Neelsen and Acid-Fast-Trichrome methods for fecal screening of Cryptosporidium parvum and Isospora belli. *Revista da Sociedade Brasileira de Medicina Tropical*, 35(3), 209-214.
- Rosypal, A., Ripley, A., Stockdale Walden, H., Blagburn, B., Grant, D., & Lindsay, D. (2012). Survival of a feline isolate of *Tritrichomonas foetus* in water, cat urine, cat food and cat litter. *Veterinary Parasitology*, 185(2-4), 279-281. Retrieved 11th October 2015 from <http://dx.doi.org/10.1016/j.vetpar.2011.11.003>
- Sabshin, S., Levy, J., Tupler, T., Tucker, S., Greiner, E., & Leutenegger, C. (2012). Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. *Journal of The American Veterinary Medical Association*, 241(3), 331-337. Retrieved 11th October 2015 from <http://dx.doi.org/10.2460/javma.241.3.331>
- Samie, A., Tsipa, M., & Bessong, P. (2013). T. *African Journal of Microbiology Research*, 7(21), 2510-2518. Retrieved 20th of February 2016 from <http://www.academicjournals.org/AJMR>

- Scorza, V., & Tangtrongsup, S. (2010). Update on the Diagnosis and Management of Cryptosporidium spp Infections in Dogs and Cats. *Topics In Companion Animal Medicine*, 25(3), 163-169. Retrieved 10th February 2016 from <http://dx.doi.org/10.1053/j.tcam.2010.07.007>
- Shaw, D., & Ihle, S. (1997). *Small animal internal medicine*. Baltimore: Williams & Wilkins.
- Shoaib, S., Hafiz, A., & Tauheed, S. (2002). Role of trichrome staining techniques in the diagnosis of intestinal parasitic infections. *JPMA. The Journal of the Pakistan Medical Association*, 52(4), 152-154.
- Skotarczak, B. (2009). Methods for parasitic protozoans detection in the environmental samples. *Parasite*, 16(3), 183-190. 11th January 2016 from <http://dx.doi.org/10.1051/parasite/2009163183>
- Stockdale, H., Dillon, A., Newton, J., Bird, R., BonDurant, R., & DeInnocentes, P. et al. (2008). Experimental infection of cats (*Felis catus*) with *Tritrichomonas foetus* isolated from cattle. *Veterinary Parasitology*, 154(1-2), 156-161. Retrieved 7th September 2015 from <http://dx.doi.org/10.1016/j.vetpar.2008.02.024>
- Stockdale, H., Givens, M., Dykstra, C., & Blagburn, B. (2009). *Tritrichomonas foetus* infections in surveyed pet cats. *Veterinary Parasitology*, 160(1-2), 13-17. Retrieve 7th September 2015 from <http://dx.doi.org/10.1016/j.vetpar.2008.10.091>
- Stockdale, H., Rodning, S., Givens, M., Carpenter, D., Lenz, S., & Spencer, J. et al. (2007). Experimental Infection of Cattle with a Feline Isolate of *Tritrichomonas Foetus*. *American Society of Parasitologica*, 93(6), 1429-1434. Retrieved 7th September from <http://www.bioone.org/doi/full/10.1645/GE-1305.1>

- Truant, A., Elliott, S., Kelly, M., & Smith, J. (1981). Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate floatation techniques for detection of intestinal parasites. *Journal Clinical Microbiology*, 13(5), 882-4.
- Tysnes, K., Gjerde, B., NÅdtvedt, A., & Skancke, E. (2011). A cross-sectional study of *Tritrichomonas foetus* infection among healthy cats at shows in Norway. *Acta Vet Scand*, 53(1), 39. Retrieved 13th January 2016 from <http://dx.doi.org/10.1186/1751-0147-53-39>
- Tyzzer, E.E., 1912. Cryptosporidium parvum (sp. nov.) a coccidium found in the small intestine of the common mouse. Archiv für Protistenkunde. 26, 394–412
- Tzannes, S., Batchelor, D., Graham, P., Pinchbeck, G., Wastling, J., & German, A. (2008). Prevalence of Cryptosporidium, Giardia and Isospora species infections in pet cats with clinical signs of gastrointestinal disease. *Journal Of Feline Medicine & Surgery*, 10(1), 1-8. Retrieved 21st November 2015 from <http://dx.doi.org/10.1016/j.jfms.2007.05.006>
- Tzannes, S., Batchelor, D., Graham, P., Pinchbeck, G., Wastling, J., & German, A. (2008). Prevalence of Cryptosporidium, Giardia and Isospora species infections in pet cats with clinical signs of gastrointestinal disease. *Journal Of Feline Medicine & Surgery*, 10(1), 1-8. Retrieved 21st November 2015 from <http://dx.doi.org/10.1016/j.jfms.2007.05.006>
- Tzipori, S., & Widmer, G. (2008). A hundred-year retrospective on cryptosporidiosis. *Trends in parasitology*, 24(4), 184-189.
- Urquhart, G., Armour, J., Duncan, J., Dunn, A., & Jennings, F. (1996). *Veterinary Parasitology* (2nd ed., pp 209-250). Glasgow: Blackwell Science

- Weitzel, T., Dittrich, S., Möhl, I., Adusu, E., & Jelinek, T. (2006). Evaluation of seven commercial antigen detection tests for Giardia and Cryptosporidium in stool samples. *Clinical Microbiology and Infection*, 12(7), 656-659. Retrieved 18th January 2016 from <http://dx.doi.org/10.1111/j.1469-0691.2006.01457.x>
- Xenoulis, P., Saridomichelakis, M., Read, S., Suchodolski, J., & Steiner, J. (2010). Detection of *Tritrichomonas foetus* in cats in Greece. *Journal of Feline Medicine & Surgery*, 12(10), 831-833. Retreived 13th January 2016 from <http://dx.doi.org/10.1016/j.jfms.2010.05.010>
- Yang, Y., & Liang, H. (2015). Prevalence and Risk Factors of Intestinal Parasites in Cats from China. *Biomed Research International*, 2015, 1-5. Retrieved 13th January 2016 from <http://dx.doi.org/10.1155/2015/967238>
- Yao, C., & Köster, L. (2015). Tritrichomonas foetus infection, a cause of chronic diarrhea in the domestic cat. *Vet Res*, 46(1), 35. Retrieved from 20th January 2016 from <http://dx.doi.org/10.1186/s13567-015-0169-0>
- Zajac, A., & Conboy, G. (2012). *Veterinary clinical parasitology*. Chichester, West Sussex, UK: Wiley-Blackwell.