



Research paper

Prevalence and molecular characterization of *Trichomonas gallinae* from domestic pigeons in Beijing, ChinaSheng-Yong Feng^{a,b,1}, Han Chang^{a,c,1}, Fu-Huang Li^{d,1}, Cheng-Min Wang^a, Jing Luo^a, Hong-Xuan He^{a,*}^a National Research Center for Wildlife-Borne Diseases, Institute of Zoology, Chinese Academy of Sciences, Chaoyang District, Beijing 100101, China^b University of Chinese Academy of Sciences, Chaoyang District, Beijing 100101, China^c College of Life Science, Hebei Normal University, Shijiazhuang 050016, China^d Beijing General Station of Animal Husbandry, Chaoyang District, Beijing 100107, China

ARTICLE INFO

Keywords:

Pigeon
T. gallinae
Beijing
Genotype

ABSTRACT

Trichomonas gallinae is a globally distributed protozoan parasite, mainly affect the upper avian digestive tract and can bring huge economic losses to pigeon industry. The objective of the present study was to determine the prevalence and genotypes of *T. gallinae* in Beijing, China. A total of 569 samples of throat swabs of pigeon were collected from pigeon farms in Shunyi District, Fangshan District, Daxing District and Miyun District of Beijing. The overall prevalence was 28.30%. The significant difference in infection rates was not observed between regions, but was found between age groups. The highest prevalence was nestling pigeons (33.16%), followed by adolescent pigeons (30.05%) and breeding pigeons (20.59%). Moreover, genotype A and B of *T. gallinae* were identified by sequencing the ITS1/5.8S/ITS2 regions and phylogenetic analysis. To our knowledge, this is the first report to display the prevalence and genotype of *T. gallinae* from Beijing, China.

1. Introduction

Avian trichomonosis, a globally parasitic disease, is caused by the protozoan *Trichomonas gallinae* (Amin et al., 2014; Forrester and Foster, 2008). The main hosts of *T. gallinae* are columbiformes. The infected birds are mainly characterized by caseous masses and ulceration in the oral cavity and other parts of upper digestive tract, which can affect feeding and even can cause starvation or suffocation (Stabler, 1947; Stabler and Herman, 1951; Borji et al., 2011). Therefore, *T. gallinae* is a major threat to pigeon industry.

The prevalence of *T. gallinae* in pigeons has been increasingly reported in the world, such as Australia (McKeon et al., 1997), Slovenia (Dovc et al., 2004), Mauritius (Gaspar Da Silva et al., 2007), Spain (Sansano-Maestre et al., 2009), Seychelles (Bunbury, 2011), Iran (Nematollahi et al., 2012), Brazil (Ecco et al., 2012), Germany (Stenkat et al., 2013), United States (Girard et al., 2014), Switzerland (Schreiber et al., 2015) and Egypt (El-Khatam et al., 2016).

In China, several reports have revealed a high prevalence in Sichuan Province (Huang et al., 2008), Guangdong Province (Luo et al., 2006; Qiu et al., 2012) and Shandong Province (Jiang et al., 2016). Moreover,

T. gallinae genotype B was identified in Guangdong Province (Qiu et al., 2017) and genotypes A, B and *Trichomonas tenax*-like strains have been discovered in Shandong Province (Jiang et al., 2016) by analysing the ITS1/5.8S/ITS2 regions respectively. Nevertheless, the data about epidemiological surveys is not available in other areas of China. Therefore, the objective of the present study was to systematically investigate the prevalence and genotypes of *T. gallinae* in Beijing, China.

2. Materials and methods

2.1. Birds sampling and study area

Oral swab samples were collected according to the previous studies (Girard et al., 2014; Rogers et al., 2016) between June and September 2017. Briefly, the oral cavity of pigeons was gently swabbed 2–3 times using a cotton-tipped applicator moistened with sterile saline, and the swab was placed into 1.5 ml centrifuge tubes equipped with tryptone/yeast extract/maltose (TYM) medium (Diamond, 1957). A total of 569 samples were collected from pigeon farms in Shunyi District, Fangshan District, Daxing District and Miyun District, where most domestic

* Corresponding author at: National Research Center for Wildlife-Borne Diseases, Institute of Zoology, Chinese Academy of Sciences, 1-5 Beichenxilu, Chaoyang District, Beijing 100101, China.

E-mail address: hehx@ioz.ac.cn (H.-X. He).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.meegid.2018.08.021>

Received 13 February 2018; Received in revised form 15 August 2018; Accepted 22 August 2018

Available online 23 August 2018

1567-1348/ © 2018 Elsevier B.V. All rights reserved.

pigeons of Beijing area are farmed. The samples were divided into 3 groups according to the age of the pigeons: nestlings (< 1 month old), adolescents (1–6 months old) and breedings (> 6 months old).

2.2. Parasite culture

All the oral swabs were transported directly to laboratory and cultured in tryptone/yeast extract/maltose (TYM) medium containing 10% fetal calf serum at 37 °C for 3 days (Diamond, 1957; Bunbury et al., 2005; Sansano-Maestre et al., 2009). All culture samples were microscopically detected by oil immersion lens (400× magnification) for the presence of *Trichomonas* Spp. Positive samples were stored in TYM medium supplemented with 5% of DMSO (Sigma-Aldrich, USA) at –80 °C for further experiment.

2.3. DNA extraction

Parasites were recovered from the positive samples by centrifugation at 800g for 5 min. Supernatant was discarded and the resulting pellet was washed three times using sterile phosphate buffered saline. Finally, the sediment was re-suspended in 200 µl of PBS. DNA was extracted with a commercial *EasyPure*® Genomic DNA kit (Beijing TransGen Biotech Co., Ltd., China). The extracted DNA was stored at –20 °C for further analysis.

2.4. PCR

The ITS1/5.8S/ITS2 sequence of the *T. gallinae* was amplified with the forward primer: TFR1 (5'-TGCTTCAGCTCAGGGTCTTCC-3') and reverse primer: TFR2 (5'-CGGTAGGTGAACCTGCCGTTGG-3') (Felleisen, 1997). PCR was carried out in 50 µl of a mixture containing 2 µl of the extracted DNA template, 10 µM of each primer, 5 µl 10 × *TransTaq*® HiFi bufferI/II, 4 µl dNTPs (2.5 mM), 1 µl *TransTaq*® HiFi DNA Polymerase and add ddH₂O to a total volume of 50 µl. A negative control was conducted with the same volume ddH₂O water in place of DNA to confirm absence of contamination. PCR was performed using the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 30 s of denaturation at 95 °C, 30 s of annealing at 58.7 °C and 30 s of extension at 72 °C. And 10 min at 72 °C for a final extension. The PCR products were detected through 1% agarose stained with GoldViewII (Solarbio, Beijing) and visualized under ultraviolet light.

2.5. Sequencing and phylogenetic analysis of the ITS1–5.8S-ITS2 region

Positive PCR products were obtained and then submitted to Liuhe Beijing Huada gene science and Technology Co., Ltd. for bidirectional sequencing. Chromatograms of the forward and reverse sequences were manually examined and assembled with Lasergene SeqMan software (DNASTAR, Madison, Wisconsin, USA). We search every sequence for the most matched sequences from GenBank by NCBI Blast and downloaded as reference sequences. Phylogenetic analysis was performed with MEGA 6.0 (Tamura et al., 2013) using the Neighbor-joining, Minimum evolution and UPGMA algorithm in a Kimura2-parameter model, and the branch reliability was analysed using 1000 bootstrap pseudo-replicates.

2.6. Statistical analysis

The chi-square test by SPSS 18.0 (SPSS Inc., IBM Corporation, Somers, NY) was used to examine the infection differences between age groups and areas. $P < 0.05$ was considered statistically significant.

2.7. Nucleotide sequence accession numbers

The sequences obtained in this study were deposited in GenBank

Table 1

Prevalence of *T. gallinae* in pigeons from Shunyi District, Fangshan District, Daxing District and Miyun District of Beijing.

Region	No. tested	No. positive	Prevalence (%)
Shunyi	155	39	25.16
Miyun	140	40	28.57
Daxing	145	45	31.03
Fangshan	129	37	28.68
Total	569	161	28.30

with accession numbers: MH733816-MH733822.

3. Results

3.1. Prevalence of *T. gallinae*

In this study, A total of 569 samples of throat swabs of pigeon were collected from pigeon farms in Shunyi District, Fangshan District, Daxing District and Miyun District of Beijing. And 161 (28.30%) were microscopically tested positive for *T. gallinae* infection. Among the positive samples, 39 (25.61%) samples were collected from Shunyi District, 40 (28.57%) samples were collected from Miyun District, 45 (31.03%) samples were collected from Daxing District and 37 (28.68%) samples were collected from Fangshan District (Table 1), separately. There was no significant difference in prevalence among the four areas ($P > 0.05$). The prevalence in different age groups ranged from 20.59% to 33.16%. The highest prevalence was found in nestling pigeons (33.16%, 65/196), followed by adolescent pigeons (30.05%, 61/203) and breeding pigeons (20.59%, 35/170). The difference in prevalence was statistically significant among different age groups ($\chi^2 = 7.57$, d. f. = 2, $P < 0.05$) (Table 2).

3.2. PCR and sequences analysis

One hundred and sixty-one samples' ITS1/5.8S/ITS2 region were successfully amplified by PCR with specific primers and obtained the fragment of around 350 bp, which was consistent with previously published studies (Felleisen, 1997). From all these PCR positive samples, fifty were randomly selected and sequenced. According to the results of sequencing, seven unique sequences were obtained and designated MH733816 (371 bp), MH733817 (341), MH733818 (341 bp), MH733819 (368 bp), MH733820 (356 bp), MH733821 (356 bp), MH733822 (343 bp), respectively. The identity of all the above sequences with the submitted sequences is $\geq 99\%$.

3.3. Phylogenetic analysis

According to previous studies, the *T. gallinae* were separated into four distinct groups on the basis of the ITS1/5.8S/ITS2 region, namely, genotype A, genotype B, genotype *T. tenax* and *T. tenax*-like group (Jiang et al., 2016). Phylogenetic tree of ITS1/5.8S/ITS2 sequences from present study and other geographic locations were constructed with the Neighbor-Joining, Minimum evolution and UPGMA method. Highly similar results, both topological structures and bootstrap values, were obtained (Fig. 1). The bootstrap values (> 70%) obtained by the three different methods were shown next to the branches, respectively.

Table 2

Prevalence of *T. gallinae* in different age groups of pigeons.

Age	No. tested	No. positive	Prevalence (%)
Nestling	196	65	33.16
Adolescent	203	61	30.05
Breeding	170	35	20.59
Total	569	161	28.30

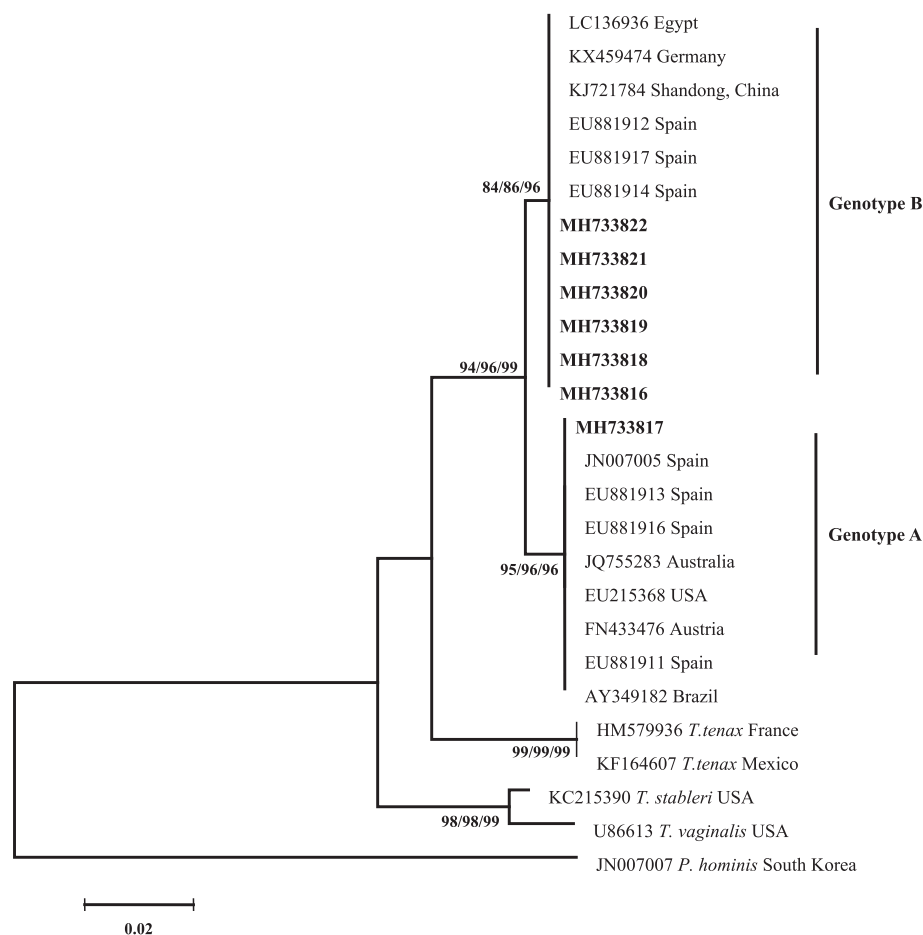


Fig. 1. Phylogenetic tree of *T. gallinae* based on ITS-1 and 5.8S rRNA and ITS2 region by the Neighbor-Joining, Minimum evolution and UPGMA method. Bootstrap method was used to test the phylogenetic tree with 1000 replications. The bootstrap values (> 70%) obtained by the three different methods were shown next to the branches respectively. *Pentatrichomonas hominis* is included as an outgroup. *T. gallinae* genotypes identified and characterized in the present study are indicated in bold-type.

All the sequences except MH733817 were clustered in the genotype B clade, while MH733817 was clustered in the genotype A clade (Fig. 1).

4. Discussion

The present study demonstrated that the infection of *T. gallinae* is common in domestic pigeon farms in Beijing, China, with the infection rates range from 25.16% to 31.03%. Previous studies have shown higher infection rate of pigeon farm in Shandong Province (33.8%, Jiang et al., 2016), Guangdong Province (33.9%, Qiu et al., 2012) and Sichuan Province (75.12%, Huang et al., 2008) in China. And detection methods, management level and sample size may be the main causes of infection deviation. The infection rate of the parasites varies greatly with the geographical location. The higher prevalence was discovered in Isfahan, Iran (57%, Nematollahi et al., 2012), in Perth, Australia (59%, McKeon et al., 1997), in the UK (60%, Lennon et al., 2013) and on the east of the Iberian Peninsula (44.8%, Sansano-Maestre et al., 2009). While much lower prevalence of *T. gallinae* was found in Egypt (1.9%, El-Khatam et al., 2016), in Spain (4.9%, Martínez-Herrero et al., 2014) and in Ljubljana, Slovenia (7.9%, Dove et al., 2004). There could be a few explanations for this situation. First, the species of *Columba* in different places are not always same. And the prevalence varied significantly among species, for example, a report conducted by Schulz et al. (2005) showed a lower prevalence (5.6%) of *Trichomonas gallinae* in Mourning doves, while a higher infection rate (50.3%) was found in Mauritian pink pigeon (Bunbury et al., 2008). Second, trichomonas can be detected by three methods: culture methods, wet-mount methods and PCR. And the sensitivity varies with methods (Ohlemeyer et al., 1998; Riley et al., 1995). Other factors could also cause the differences, such as climate difference, management level and sample size.

Unlike in Shandong province, China, where the highest infection rate was detected in adolescent pigeons and the lowest infection rate was found in nestling group (Jiang et al., 2016), the present study showed that the prevalence of *T. gallinae* in nestling pigeons was significantly higher than adolescent pigeons. This result was consistent with the previous studies from South Khorasan in Iran and from Min-oufiya in Egypt, where the infection rates between nestling pigeons and adolescent pigeons were 93.18% vs 31.03% (Radfar et al., 2012) and 2.13% vs 1.87% (El-Khatam et al., 2016), respectively. The higher prevalence in nestling pigeons probably owing to some reasons: First, the nestlings are fed crop milk from infected adults. Second, infection can occur through contaminated food with the parasites in feeders and water. Third, the resistance of pigeons to infection with *T. gallinae* may increase with age.

The seven unique sequences obtained in this study were divided into two branches by phylogenetic analysis. MH733816, MH733818, MH733819, MH733820, MH733821 and MH733822 together with LC136936 (Egypt), KX459474 (Germany), KJ721784 (Shandong, China), EU881912 (Spain), EU881917 (Spain), EU881914 (Spain) were clustered in genotype B group, while MH733817 along with 4 isolates from Spain (JN007005, EU881913, EU881916 and EU881911), 2 isolates from Australia (JQ755283 and FN433476), 1 isolate from USA (EU215368) and 1 isolate from Brazil (AY349182) were clustered in genotype A group. Consistent with previous studies that the pathogenicity of genotype A is stronger compared with genotype B (Martínez-Herrero et al., 2014; McBurney et al., 2015), the genotype A isolated in our study was recovered from pigeons with oral lesions. Though the majority of the pigeons infected with genotype B were seemingly healthy, severe pigeons with ulceration in the oral cavity were also observed. This result was in line with the study of Sansano-Maestre

et al. (2009). Therefore, further studies to find out the relationship between pathogenicity and genotype of *Trichomonas* is certainly needed.

5. Conclusions

In conclusion, the present survey first reports the prevalence and genotypes of *Trichomonas* spp. in domestic pigeons in Beijing, China. The average infection rate is 28.30% and the highest prevalence was discovered in nestling pigeons. Furthermore, both genotype A and B were identified in domestic pigeons in our studies. These findings enrich the epidemiological data of *T. gallinae* infection and are useful for further research on the molecular epidemiology and control of *T. gallinae* infection in poultry in China.

Conflict of interest

The authors have declared that no competing interests.

Acknowledgments

This work was supported by grants from the China Agriculture Research System Poultry-related Science and Technology Innovation Team of Peking (CARS-PSTP) and the Wildlife-Borne Disease Surveillance Program from the State Forestry Administration of the People's Republic of China (CZBZX-1).

References

- Amin, A., Bilic, I., Liebhart, D., Hess, M., 2014. Trichomonads in birds—a review. *Parasitology* 141, 733–747.
- Borji, H., Razmi, G.H., Movassaghi, A.H., Moghaddas, E., Azad, M., 2011. Prevalence and pathological lesion of *Trichomonas gallinae* in pigeons of Iran. *J. Parasit. Dis.* 35, 186–189.
- Bunbury, N., 2011. Trichomonad infection in endemic and introduced columbids in the Seychelles. *J. Wildl. Dis.* 47, 730–733.
- Bunbury, N., Bell, D., Jones, C., Greenwood, A., Hunter, P., 2005. Comparison of the InPouch TF culture system and wet-mount microscopy for diagnosis of *Trichomonas gallinae* infections in the Pink Pigeon *Columba mayeri*. *J. Clin. Microbiol.* 43, 1005–1006.
- Bunbury, N., Jones, C.G., Greenwood, A.G., Bell, D.J., 2008. Epidemiology and conservation implications of *Trichomonas gallinae* in the endangered Mauritian pink pigeon. *Biol. Conserv.* 141, 153–161.
- Diamond, L.S., 1957. The establishment of various *Trichomonas* of animals and man in axenic cultures. *J. Parasitol.* 43, 488–490.
- Dovc, A., Zorman-Rojs, O., Vergles Rataj, A., Bole-Hribovsek, V., Krapez, U., Dobeic, M., 2004. Health status of free-living pigeons (*Columba livia domestica*) in the city of Ljubljana. *Acta Vet. Hung.* 52, 219–226.
- Ecco, R., Preis, I.S., Vilela, D.A., Luppi, M.M., Malta, M.C., Beckstead, R.B., Stimmelmayer, R., Gerhold, R.W., 2012. Molecular confirmation of *Trichomonas gallinae* and other parabasalids from Brazil using the 5.8S and ITS-1 rRNA regions. *Vet. Parasitol.* 190, 36–42.
- El-Khatam, A.O., Aboulaila, M.R., Ibrahim, M., Abdel-Gaber, M.M., 2016. *Trichomonas gallinae*: prevalence and molecular characterization from pigeons in Minoufiya governorate, Egypt. *Exp. Parasitol.* 170, 161–167.
- Felleisen, R.S., 1997. Comparative sequence analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. *Parasitology* 115, 111–119.
- Forrester, D.J., Foster, G.W., 2008. *Parasitic Diseases of Wild Birds*. Blackwell Publishing, Ames, Iowa.
- Gaspar Da Silva, D., Barton, E., Bunbury, N., Lunness, P., Bell, D.J., Tyler, K.M., 2007. Molecular identity and heterogeneity of trichomonad parasites in a closed avian population. *Infect. Genet. Evol.* 7, 433–440.
- Girard, Y.A., Rogers, K.H., Woods, L.W., Chouicha, N., Miller, W.A., Johnson, C.K., 2014. Dual-pathogen etiology of avian trichomonosis in a declining band-tailed pigeon population. *Infect. Genet. Evol.* 24, 146–156.
- Huang, M.Y., Liao, D.J., Wen, H., Fu, A.J., 2008. A report on *Trichomonas gallinae* in pigeons in Sichuan Province of China. *Chin. Poult.* 30, 56–57 (In Chinese).
- Jiang, X., Sun, J., Wang, F., Li, H., Zhao, X., 2016. Prevalence of *Trichomonas* spp. in domestic pigeons in Shandong Province, China, and genotyping by restriction fragment length polymorphism. *Vet. J.* 211, 88–93.
- Lennon, R.J., Dunn, J.C., Stockdale, J.E., Goodman, S.J., Morris, A.J., Hamer, K.C., 2013. Trichomonad parasite infection in four species of Columbidae in the UK. *Parasitology* 140, 1368–1376.
- Luo, F., Weng, Y.B., Zhang, J.F., Li, G.Q., 2006. A survey of *Trichomonas gallinae* in pigeons in Guangdong Province of China. *Chin. Poult.* 28, 24–25 (In Chinese).
- Martínez-Herrero, M.C., Sansano-Maestre, J., López Márquez, I., Obón, E., Ponce, C., González, J., Garijo-Toledo, M.M., Gómez-Muñoz, M.T., 2014. Genetic characterization of oropharyngeal trichomonad isolates from wild birds indicates that genotype is associated with host species, diet and presence of pathognomonic lesions. *Avian Pathol.* 43, 535–546.
- McBurney, S., Kelly-Clark, W.K., Forzán, M.J., Lawson, B., Tyler, K.M., Greenwood, S.J., 2015. Molecular characterization of *Trichomonas gallinae* isolates recovered from the Canadian Maritime provinces' wild avifauna reveals the presence of the genotype responsible for the European finch trichomonosis epidemic and additional strains. *Parasitology* 142, 1053–1062.
- McKeon, T., Dunsmore, J., Raidal, S.R., 1997. *Trichomonas gallinae* in budgerigars and columbid birds in Perth, Western Australia. *Aust. Vet. J.* 75, 652–655.
- Nematollahi, A., Ebrahimi, M., Ahmadi, A., Himan, M., 2012. Prevalence of *Haemoproteus columbae* and *Trichomonas gallinae* in pigeons (*Columba domestica*) in Isfahan, Iran. *J. Parasit. Dis.* 36, 141–142.
- Ohlemeyer, C.L., Hornberger, L.L., Lynch, D.A., Swierkosz, E.M., 1998. Diagnosis of *T. vaginalis* in adolescent females: inPouch TV culture versus wet-mount microscopy. *J. Adolesc. Health* 22, 205–208.
- Qiu, S.B., Lv, M.N., He, X., Weng, Y.B., Zou, S.S., Wang, X.Q., Lin, R.Q., 2017. PCR identification and phylogenetic analysis of *Trichomonas gallinae* from domestic pigeons in Guangzhou, China. *Korean J. Parasitol.* 55, 333–336.
- Qiu, S.B., Yan, C., Zhou, D.H., Hou, J., Wang, Q.Q., Lin, Y., Fu, H.C., Zhang, J., Weng, Y.B., Song, H.Q., Lin, R.Q., 2012. High prevalence of *Trichomonas gallinae* in domestic pigeons (*Columba livia domestica*) in subtropical southern China. *African J. Microbiol. Res.* 6, 3261–3264.
- Radfar, M.H., Asl, E.N., Seghinsara, H.R., Dehaghi, M.M., Fathi, S., 2012. Biodiversity and prevalence of parasites of domestic pigeons (*Columba livia domestica*) in a selected semiarid zone of South Khorasan, Iran. *Trop. Anim. Health Prod.* 44, 225–229.
- Riley, D.E., Wagner, B., Polley, L., Krieger, J.N., 1995. PCR-based study of conserved and variable DNA sequences of *Trichomonas foetus* isolates from Saskatchewan. *Canada J. Clin. Microbiol.* 33, 1308–1313.
- Rogers, K.H., Girard, Y.A., Woods, L., Johnson, C.K., 2016. Avian trichomonosis in spotted owls (*Strix occidentalis*): Indication of opportunistic spillover from prey. *Int J Parasitol Parasites Wildl.* 5, 305–311.
- Sansano-Maestre, J., Garijo-Toledo, M.M., Gómez-Muñoz, M.T., 2009. Prevalence and genotyping of *Trichomonas gallinae* in pigeons and birds of prey. *Avian Pathol.* 38, 201–207.
- Schreiber, T., Kamphausen, L., Haag-Wackernagel, D., 2015. Effects of the environment on health of feral pigeons (*Columba livia*). *Berl. Munch. Tierarztl. Wochenschr.* 128, 46–60.
- Schulz, J.H., Bermudez, A.J., Millsbaugh, J.J., 2005. Monitoring presence and annual variation of trichomoniasis in mourning doves. *Avian Dis.* 49, 387–389.
- Stabler, R.M., 1947. *Trichomonas gallinae*, pathogenic trichomonad of birds. *J. Parasitol.* 33, 207–213.
- Stabler, R.M., Herman, C.M., 1951. Upper digestive tract trichomoniasis in mourning doves and other birds. *Trans. North. Am. Wildlife Conf.* 16, 145–163.
- Stenkat, J., Krautwald-Junghans, M.E., Schmidt, V., 2013. Causes of morbidity and mortality in free-living birds in an urban environment in Germany. *EcoHealth* 10, 352–365.
- Tamura, K., Stecher, G., Peterson, D., Filipiński, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729.