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Genotypic diversity and epidemiology of *Trichomonas gallinae* in Columbidae: Insights from a comprehensive analysis

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ABSTRACT

Trichomonas gallinae is a protozoa that parasitizes the upper gastrointestinal and respiratory tracts of various animals and birds, including Columbidae, Passeriformes, and Falconiformes. Polymerase chain reaction-based *T. gallinae* ITS1/5.8S/ITS2 gene typing yields inconsistent results owing to methodological differences. To standardize the statistical analysis of *T. gallinae* genotype distributions, this study employed MEGA-X software with the Tamamura 3-parameter (T92) + G model in the neighbor-joining method, with 2,000 bootstrap replicates, to calculate a systematic evolutionary tree. The resulting tree comprised 12 branches, ITS-OBT-Tg-1 to ITS-OBT-Tgl, with similar phylogenetic relationships. Relevant literature review yielded *T. gallinae* prevalence data in Columbidae. Statistical analysis was conducted from two perspectives: non-biological and biological factors, using chi-square tests and ordered logistic regression analysis. *T. gallinae* positivity rates differed significantly across diverse regions ($\chi^2 = 4{,}609.9$, $P = 0.000$, $df = 4$) and at various times ($\chi^2 = 2{,}810.8$, $P = 0.000$, $df = 3$). However, temperature and precipitation did not significantly affect *T. gallinae* positivity rates. Additionally, *T. gallinae* positivity rates differed significantly among diverse hosts ($\chi^2 = 2{,}958.6$, $P = 0.000$, $df = 14$) and by host age ($\chi^2 = 478.5$, $P = 0.000$, $df = 2$) and sex ($\chi^2 = 96.00$, $P = 0.000$, $df = 1$). This comprehensive analysis aimed to control *T. gallinae* transmission, reduce economic and species resource losses, and provide a foundation for future related research.

1. Introduction

Trichomonas gallinae is a protozoan that mainly infects birds such as Columbiformes, Passeriformes, and Falconiformes (Santos et al., 2019). It affects the upper digestive and respiratory tracts and potentially causes various levels of clinical symptoms (Rogers et al., 2018). As a result, it can have significant implications for avian populations. In particular, *T. gallinae* infections can manifest as mild, moderate, or severe based on clinical symptoms, with mild infections which are often asymptomatic in pigeons (Martínez-Herrero et al., 2020). The consequential damage caused by *T. gallinae* infections emphasizes the need for a thorough understanding of the disease's prevalence characteristics among Columbidae, especially in regions heavily affected by this protozoan, such as Colombia (Qamar et al., 2021). In our study, we aimed to address this gap by presenting a comprehensive analysis of avian trichomonosis in Colombia.

Clinically, polymerase chain reaction (PCR) analysis is frequently utilized for the detection and typing of the ITS1/5.8S/ITS2 gene sequence of *T. gallinae* (Brunthaler et al., 2022; El-Khatam et al., 2016). However, it is important to note that the variable lengths of the PCR products currently used for sequencing could introduce variability in subsequent genotyping results. This variability in product length

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Table 1
Genotypes of the ITS1/5.8SS/ITS2 region of oral *Trichomonas* sp. isolated from birds described by other authors and genetic group codification used in this paper (ITS-OBT-Tx).

Gerhold et al. (2008)		Marx et al. (2017)		Grabensteiner et al. (2010)		Martínez-Herrero et al. (2014)		Chi et al. (2013)		This paper	
Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences
A	EU215369	A/B	JN007005			ITS-OBT-Tg-1	AY349182	A	GQ150752	ITS-OBT-Tg-1.1	JN007005
							EF208019			ITS-OBT-Tg-1.2	EU215369
							EU215368			ITS-OBT-Tg-1.3	FN433476
							EU215369			ITS-OBT-Tg-3	KX459442
							EU290649	B	EU215368	ITS-OBT-Tg-4	AY349182
							FN433476			ITS-OBT-Tg-5	GQ150752
							HG008050			ITS-OBT-Tg-6	EU215368
							KC215387			ITS-OBT-Tg-7	MH459331
							GQ150752				
							EU881911				
							EU881913				
							EU881915				
							EU881916				
B	EU215368	C/V/N	KX459485			ITS-OBT-Tg-2	EU215362	C	EU215362	ITS-OBT-Tg-2.1	FN433477
							EU881912, 14,	E	EU215363	ITS-OBT-Tg-2.2	EU215363
				I	U86614		17	D	EU215364	ITS-OBT-Tg-2.3	EU215364
					EU215362		EU215363-64			ITS-OBT-Tg-2.4	FN433475
					FN433475		FN433475				
				V	FN433477		FN433477	V	FN433477	ITS-OBT-Tg-2.5	EU215362
							U86614				
C	EU215362					ITS-OBT-Ts-1	EU215367	F	EU215358	ITS-OBT-Tsl-1	EU215367
							JX089392				
							KC215389				
							KC215390				
						ITS-OBT-Tsl-2	EU215358	G	EU215359		
D	EU215364					ITS-OBT-*Trichomonas sp.*-1	FN433473	H	EU215360	ITS-OBT-*Trichomonas sp.*-1.1	KX459510
						ITS-OBT-*Trichomonas sp.*-2	KC529665	I	EU215361	ITS-OBT-*Trichomonas sp.*-1.2	KY680342
										ITS-OBT-*Trichomonas sp.*-2	JX512969
E	EU215363					ITS-OBT-*Trichomonas sp.*-3	EU215359	J	EU215365	ITS-OBT-*Trichomonas sp.*-3	EU215359
										ITS-OBT-*Trichomonas sp.*-4	FN433473
										ITS-OBT-*Trichomonas sp.*-5	JQ692128
						ITS-OBT-Tvl-1	EU215361	L	EU215366	ITS-OBT-Tvl-1	EU215360
											EU215361
F	EU215358					ITS-OBT-Tvl-2	EU215366	K	EU215367	ITS-OBT-Tvl-2	AY349185
G	EU215359	O	KX459442			ITS-OBT-Tvl-3	EU215360			ITS-OBT-Tvl-3	EU215366
H	EU215360	Q	KX459510			ITS-OBT-Tvl-4	EU215365			ITS-OBT-Tvl-4	EU215365
I	EU215361	R	MH459331			ITS-OBT-Tvl-5	FN433478				
							KF214774				
							KF993707				
J	EU215365	P	KF993705			ITS-OBT-Ttl-1	FN433474			ITS-OBT-Ttl-1	FN433474
										ITS-OBT-Ttl-2	JQ755288
K	EU215367			II	FN433474	ITS-OBT-Tcl-1	KF993705	II	FN433474	ITS-OBT-Tcl-1	KF993705
L	EU215366	III	KC529665	III	FN433473	ITS-OBT-Tcl-2	KF993706	III	FN433473	ITS-OBT-Tcl-2	KF993706
											KY767988

(*continued on next page*)

Table 1 (*continued*)

Gerhold et al. (2008)		Marx et al. (2017)		Grabensteiner et al. (2010)		Martínez-Herrero et al. (2014)		Chi et al. (2013)		This paper	
Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences	Genotype group	Genebank sequences
				VI	EU215359 EU215360 EU215361 EU215363 AY349185 AY871048 AY244652 U86615 FN43478	ITS-OBT-*Simplicimonas. sp.*	JX089388	VI	FN433478	ITS-OBT-Tgl-1	FN433478
				IV	AY349182 EU215360 FN433476	ITS-OBT-*Trichomonadida spp.*	EU290650			ITS-OBT-*Trichomonas sp.*-6 ITS-OBT-*Trichomonas sp.*-7	JQ755273 JX512968

presented challenges in achieving consistent genotyping outcomes, not due to the PCR detection itself, but rather due to the diverse product sizes used in subsequent sequencing.

In this study, we presented a comprehensive analysis of avian trichomonosis in Colombia, one of the hosts most affected by *T. gallinae*. Based on a synthesis of all existing reported articles on the prevalence characteristics of *T. gallinae* among Columbidae, the chi-square test and logistic ordered regression analysis were used to explore all possible influencing factors. Our study aimed to enhance current understanding of the prevalence of *T. gallinae* in avian populations, its impact on bird health, and possible prevention and management strategies for the disease.

2. Materials and methods

2.1. Article screening and data collection

A total of 281 search results collected in place of generated from databases, including Science Citation Index (SCI) and PubMed, using keywords such as "*Trichomonas gallinae*", "infection" and "genotype". Searching for keywords such as "pigeon trichomoniasis" and "infection rate" yielded 85 results. We screened relevant articles on the prevalence of *T. gallinae* in Columbidae and the genotyping of *T. gallinae* based on the ITS gene sequence, resulting in a total of 43 articles. Among them, 36 articles were used for statistical analysis of the prevalence of *T. gallinae* infection. Information regarding the articles included in each database were shown in Table S1, Table S2, and Fig. S1. Some reference articles appeared in multiple databases; therefore, the total number of articles analyzed in the table exceeded 366.

2.2. Evolutionary tree construction

All sequences for tree construction were obtained from GeneBank. The neighbor-joining algorithm with 2,000 replications was applied using MEGA-X software with Tamamura 3-parameter (T92) + G model (Chou et al., 2022), with *P. hominis* (AF1569641) as an outgroup.

2.3. Statistical analysis

The infection rates of *T. gallinae* in different regions, hosts, sexes, ages, and times were expressed as rates. The 95% CI of each rate was calculated using "The Confidence Interval of a Proportion" model on the VassarStats website. Rate comparison was conducted using the Crosstabs calculation model in SPSS.R24.0.0.0 software. The statistical analysis results were expressed in terms of the chi-square (χ^2) value, P value, and degree of freedom (df). The χ^2 value represented the correlation analysis between two or more sample rates. The P value indicated the significance of the differences between sample rates. $P < 0.01$ indicated an extremely significant difference, $0.01 < P < 0.05$ indicated a significant difference, and $P > 0.05$ indicated a non-significant difference. The df value represented the number of unrestricted variables used to calculate statistical measures. The correlation analysis between the positivity rate of *T. gallinae* and climatic factors (temperature and precipitation) was conducted using ordered logistic regression analysis in SPSS.R24.0.0.0 software. The statistical analysis results were expressed in terms of standard error of the mean (SEM), regression coefficient (β), OR, P-value (P), and 95% CI for each OR. The SEM value represented the variation between sample means, and the smaller the value, the more representative the sample statistics were of the population parameter.

3. Results

3.1. Phylogenetic analysis of Trichomonas based on ITS1/5.8S/ITS2 sequences

The tree was constructed with 223 bp of aligned nucleotide positions

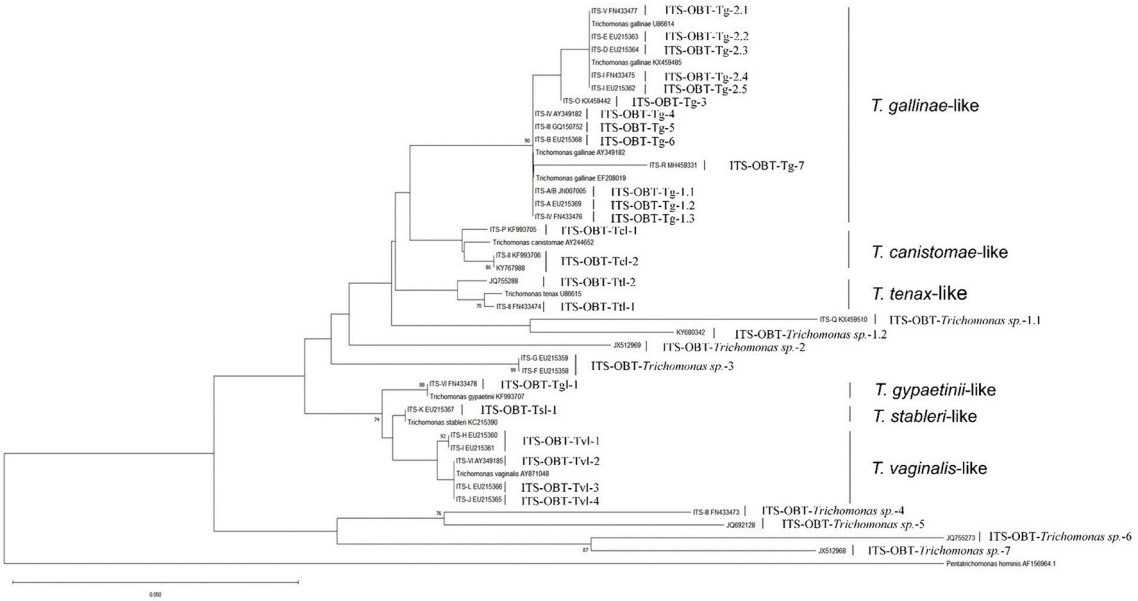

Fig. 1. Systematic phylogenetic tree based on *Trichomonas*.sp ITS1/5.8S/ITS2 gene typing. The length of ITS1/5.8S/ITS2 was 223bp. This tree was generated using MEGA-X software and calculated using the Tamamura 3-parameter (T92) + G model and the neighbor-joining method with 2,000 bootstrap replicates. Bootstrap values < 70% were not shown.

of ITS1/5.8S/ITS2 using MEGA-X software via a neighbor-joining algorithm with 2,000 replications in the Tamamura 3-parameter (T92) + G model, with *Pentatrichomonas hominis* (AF1569641) as an outgroup. In this study, 43 *Trichomonas* ITS1/5.8S/ITS2 sequences uploaded to the National Center for Biotechnology Information database were used to construct a systematic phylogenetic tree. The reference sequences for each branch were shown in Table 1.

In the tree (Fig. 1), the *T. gallinae*-like group was most closely related to *T. gallinae* (U86614, KX459485, AY349182, and EF208019) and could be considered to contain 13 genotypes. To enhance the clarity of the evolutionary depiction within the *T. gallinae* system, we undertook a systematic renaming of existing genotypes. ITS-A/B (JN007005) was renamed ITS-OBT-Tg-1.1, ITS-A (EU215369) was renamed ITS-OBT-Tg-1.2, ITS-IV (FN433476) was renamed ITS-OBT-Tg-1.3, ITS-V (FN433477) was renamed ITS-OBT-Tg-2.1, ITS-E (EU215363) was renamed ITS-OBT-Tg-2.2, ITS-D (EU215364) was renamed ITS-OBT-Tg-2.3, ITS-I (FN433475) was renamed ITS-OBT-Tg-2.4, ITS-I (EU215362) was renamed ITS-OBT-Tg-2.5, ITS-O (KX459442) was renamed ITS-OBT-Tg-3, ITS-IV (AY349182) was renamed ITS-OBT-Tg-4, ITS-III (GQ150752) was renamed ITS-OBT-Tg-5, ITS-B (EU215368) was

Table 2
T. gallinae positive rates of Columbidae in different countries.

Continent	Country	Positive rate% (Positive number/sample size)	95%CI	References
North America	USA	5.5 (239/4341)	4.86-6.24	(Girard et al., 2014, Glass et al., 2001, Schulz et al., 2005)
	Canada	2.0 (1/49)	0.11–12.24	Mcburney et al. (2015)
Total		5.5 (240/4390)	4.82-6.19	
Europe	UK	51.97 (66/127)	42.97-60.85	(Lennon et al., 2013, Shopland et al., 2021, Stockdale et al., 2014)
	Spain	48.1 (381/792)	44.59-51.65	(Martínez-Herrero et al., 2014, Marx et al., 2017, Santos et al., 2019, Villanúa et al., 2006)
	Germany	75.7 (178/235)	69.65–80.97	(Marx et al., 2017, Quillfeldt et al., 2018)
	Malta	25.0 (9/36)	12.73-42.54	
	Italy	80.0 (16/20)	55.73–93.39	
	Poland	37.0 (37/100)	27.73-47.29	(Bobrek et al., 2017)
	Slovenia	7.9 (11/139)	4.21-14.05	(Dovč et al., 2004)
	Ireland	15.0 (3/20)	3.96-38.86	(Doyle et al., 2022)
Total		47.7 (701/1469)	45.14–50.31	
Asia	China	56.7 (3848/6789)	55.49–57.86	(Cai et al., 2022, Feng et al., 2018, Jiang et al., 2016, Qiu et al., 2012, Qiu et al., 2017, Huang et al., 2009, Sun et al., 2020, Zhang, 2009)
	Iraq	12.6 (63/499)	9.91-15.94	(Alasadiy et al., 2022, Wahhab et al., 2017)
	Iran	47.3 (510/1078)	44.30–50.34	(Adinehbeigi et al., 2018, Borji et al., 2011, Nematollahi et al., 2012)
	India	26.9 (87/324)	22.17–32.09	(Saikia et al., 2021)
	Turkey	22.2 (4/18)	7.37–48.08	(Stimmelmayr et al., 2012)
	Bangladesh	66.1 (238/360)	60.93-70.94	(Arfin et al., 2019, Begum et al., 2008)
Total		52.4 (4750/9068)	51.35–53.41	
Africa	Seychelles	31.7 (38/120)	23.65-40.88	(Bunbury, 2011)
	Libya	55.0 (55/100)	44.75-64.86	(Alkharigy et al., 2018)
	Mauritius	44.3 (131/296)	38.55-50.12	Bunbury et al. (2007)
	Sudan	16.3 (49/300)	12.45-21.11	Mohamed (2015)
	Egypt	2.8 (95/3365)	2.30-3.45	(El-Khatam et al., 2016, Mohamed et al., 2023)
Total		8.8 (368/4181)	7.97-9.71	
Oceania	Australia	76.5 (247/323)	71.39–80.91	(Peters et al., 2020)
Overall		32.5 (6306/19431)	31.79-33.11	

renamed ITS-OBT-Tg-6, and ITS-R (MH459331) was renamed ITS-OBT-Tg-7. ITS-K (EU215367) and *T. stableri* (KC215390) were on the same branch and were renamed ITS-OBT-Tsl-1. ITS-P (KF993705) and ITS-II (KF993706 and KY767988) were classified into the *T. canistomae*-like group and renamed ITS-OBT-Tcl-1 and ITS-OBT-Tcl-2, respectively. They were most closely related to *T. canistomae* (AY244652).

All sequences in the *T. vaginitis*-like group were most closely related to *T. vaginitis* (AY871048). ITS-H (EU215360) and ITS-I (EU215361) were renamed ITS-OBT-Tvl-1, ITS-VI (AY349185) was renamed ITS-OBT-Tvl-2, ITS-L (EU215366) was renamed ITS-OBT-Tvl-3, and ITS-J (EU215365) was renamed ITS-OBT-Tvl-4. ITS-II (FN433474) and JQ755288 were more closely related to *T. tenax* (U86615) and were renamed ITS-OBT-Ttl-2 and ITS-OBT-Ttl-1, respectively. ITS-VI (FN433478) was on the same branch as *T. gypaetinii* (KF993707) and was renamed ITS-OBT-Tgl-1. KX459510, KY680342, JX512969, EU215359, EU215358, FN433473, JQ692128, JQ755273, and JX512968 were not closely related to any other species on the tree and were classified as ITS-OBT-*Trichomonas* sp. (n).

3.2. Epidemiological characteristic analysis of *T. gallinae* infection in Columbidae

3.2.1. Non-biological factors driving infection

3.2.1.1. Worldwide prevalence of T. gallinae infection in Columbidae. The overall positivity rate of *T. gallinae* infection in Columbidae worldwide was 32.5%, with positivity rates ranging from 2.0% to 80.0% across different countries (Table 2). Canada had the lowest positivity rate (2.0%), while Italy had the highest (80.0%). Statistical analysis revealed a significant difference in the positivity rates of *T. gallinae* infection in Columbidae among various countries ($\chi^2 = 5{,}751.7$, $P = 0.000$, $df = 21$). The positivity rates in North America, Europe, Asia, Africa, and Oceania were 5.5%, 47.7%, 52.4%, 8.8%, and 76.5%, respectively. Statistical analysis of the positivity rates of *T. gallinae* infection in Columbidae among different continents revealed a significant difference ($\chi^2 = 4{,}609.9$, $P = 0.000$, $df = 4$). The positivity rates also varied among different countries within each continent. In North America, Europe, Asia, and Africa, the United States (5.5%), Italy (80.0%), Bangladesh (66.1%), and Libya (55.0%) had the highest positivity rates, respectively.

3.2.1.2. Temporal trends in prevalence. This study comprehensively analyzed all reported years of *T. gallinae* infection in Columbidae, and the results were presented in Fig. S2 and Table S3. From 1998 to 2021, the positivity rate of *T. gallinae* exhibited a parabolic trend, with the highest positivity rate of 61.6% occurring in 2004–2009. The χ^2 test showed a significant difference in positivity rates among various time periods ($\chi^2 = 2{,}810.8$, $P = 0.000$, $df = 3$). In Table S4, the β value for "year" was 0.066, and the odds ratio (OR) was 1.07, indicating that time was a risk factor for *T. gallinae* infection, and the infection rate may increase with time. However, the P value was 0.147, indicating no significant difference. Thus, although an increase in positivity rates is possible in the future, no statistical difference existed compared with past rates.

3.2.1.3. Climate-related prevalence characteristics. To investigate the association of the positivity rate of *T. gallinae* infection in Columbidae with temperature and precipitation, monthly average temperature and precipitation data from the reported areas were consulted. An ordered multiclass logistic regression analysis was performed to establish a correlation model between temperature and the *T. gallinae* positivity rate, and the results were shown in Table S5. The OR value of temperature was 1.07, indicating that temperature was a risk factor for the *T. gallinae* positivity rate. However, since the P value exceeded 0.05 ($P = 0.168$), the effect of temperature on the positivity rate was not significant. The OR value of precipitation was 1.00, indicating that precipitation had no effect on the *T. gallinae* positivity rate. However, since the P value was 0.539, precipitation did not cause a significant difference. When the temperature was within the 15–32 °C range, the positivity rate decreased as temperature increased; however, when the temperature was <15 °C, the positivity rate exhibited an increasing trend with rising temperature (Fig. S3). Nevertheless, owing to the limited data for this period, the trend was not statistically significant. As precipitation increased within <100 mm, it displayed a decreasing trend in the positivity rate; nonetheless, when precipitation exceeded 100 mm, the data sample was too small to be statistically significant.

3.2.2. Biological factors

3.2.2.1. Host-species characteristics. To proceed with the analysis, groups with a small sample size ($n < 10$) and a 95% confidence interval (CI) deviation greater than 50% were excluded from the statistical analysis. This exclusion ensured that the results obtained were statistically robust. After excluding certain species from the analysis, we conducted a chi-square test on the remaining species with sufficient sample sizes and identified an extremely significant difference in the positivity rate among species ($\chi^2 = 2{,}958.6$, $df = 14$, $P = 0.000$). Besides the genera excluded from the analysis, the genus with the highest positivity rate was *Otidiphaps*, which had an infection rate of 100% (12/12, 95% CI = 75.75–100.00). The genus with the lowest positivity rate was *Zenaida*, which had an infection rate of 5.5% (227/4105). Positivity-rate differences among the various Columbidae genera were extremely significant ($\chi^2 = 2{,}634.8$, $df = 8$, $P = 0.000$). Susceptibility to *T. gallinae* infection also varied among different species within each genus. For example, the infection rate of *Columba fasciata* was relatively low, at only 4.3%, while that of *Columba oenas* was considerably higher, at 83.5%, and the difference was extremely significant according to statistical analysis ($\chi^2 = 237.9$, $df = 3$, $P = 0.000$).

3.2.2.2. Age-related prevalence characteristics. To further understand the prevalence characteristics of *T. gallinae* infection in Columbidae, we collected data on the age of infected birds from various countries. As shown in Table S7, the highest prevalence was found in young pigeons aged 30–180 days, with a prevalence of 47.3%, while the lowest prevalence was observed in adult pigeons older than 180 days, with a prevalence of 28.5%. Statistical analysis revealed significant differences in infection rates among different age groups ($\chi^2 = 478.5$, $df = 2$, $P = 0.000$). These results suggested that the prevalence of T. gallinae infection in Columbidae varied significantly with age. Additionally, we observed variations in infection rates among different age groups across regions, as detailed in Table S7.

3.2.2.3. Sex-related prevalence characteristics. In addition to the factors mentioned above, we also examined the effect of sex on the *T. gallinae* positivity rate in Columbidae. As shown in Table S8, the positivity rate in male birds was 34.3%, while that in female birds was 54.8%. Statistical analysis revealed a significant difference in *T. gallinae* positivity rates between male and female Columbidae ($\chi^2 = 96.00$, $P = 0.000$, $df = 1$).

4. Discussion

The ITS1/5.8S/ITS2 gene typing of *T. gallinae* has been labeled differently in the literature, with three main nomenclature approaches. The first approach, introduced by Gerhold et al. (2008), employed designations such as Genotype A, B, C, D, and E. The second approach, proposed by Martínez-Herrero et al. (2014), employed names like ITS-OBT-Tg-1 and ITS-OBT-Tg-2. The third approach, presented by Grabensteiner et al. (2010), used designations like ITS-I and ITS-II. Marx et al. (2017) referred to the lineages from Gerhold's work, naming

lineage C/V/N as "genotype A″ and lineage A/B as "genotype B″, introducing lineages O, P, and Q. There was disagreement in naming sequences; for example, the sequence EU215359 had been referred to as "G" by Gerhold, "VI" by Grabensteiner, and as "ITS-OBT-Trichomonas sp.-3″ by Martínez-Herrero. This inconsistency in naming poses challenges for evolutionary relationship studies within avian trichomonads. To address this, we conducted a comprehensive analysis and constructed a phylogenetic tree, laying a foundation for future research on avian trichomonads.

Based on the evolutionary tree established, the dominant genotypes of *T. gallinae* across different countries and hosts were identified as ITS-OBT-Tg-1 and ITS-OBT-Tg-2, with occasional occurrences of other genotypes. ITS-OBT-Tg-1 is known for its substantial virulence, prevalent in carnivores, Columbiformes, Passeriformes, and Psittaciformes (Martínez-Herrero et al., 2014). Conversely, ITS-OBT-Tg-2 exhibits weaker virulence and is predominant in raptors and Columbidae (McBurney et al., 2015). Additionally, a potential relationship between *T. vaginalis*, a common human sexually transmitted parasitic infection, and Trichomonas species from pigeons in the United States has been explored. Trichomonas species from pigeons are believed to be the ancestors of vaginal trichomoniasis, with the ITS-OBT-Tvl genotype identified as a potential origin of *T. vaginalis* (Peters et al., 2020). The ITS-OBT-Tvl genotype was reported in *Patagioenas fasciata*, *Zenaida asiatica*, *Streptopelia decaocto*, *Zenaida macroura*, *Accipiter cooperii* (Gerhold et al., 2008), and *Gypaetus barbatus* (Grabensteiner et al., 2010). It was considerably homologous to *T. vaginalis* (AY433478) found in humans; nevertheless, no association existed with macroscopic lesions in birds. Other genotypes, such as ITS-OBT-Ttl and ITS-OBT-Tcl, exhibit genetic relationships with *T. tenax* (Grabensteiner et al., 2010) and *T. canistomae* (Kutisova et al., 2005), respectively. ITS-OBT-Ttl was first discovered in *C. livia* forma domestica, while the ITS-OBT-Tcl genotype was initially reported in European turtle doves (*Streptopelia turtur*) and *Accipiter gentilis*. However, their pathogenicity remains uncertain, with no evidence of macroscopic lesions on the host. Similarly, the ITS-OBT-Trichomonas.sp1. and ITS-OBT-Trichomonas.sp4. genotypes, first identified in *S. turtur* and *C. livia* forma domestica respectively, have been found not to cause clinical symptoms or macroscopic lesions in their hosts (Marx et al., 2017). Epidemiological data suggest a correlation between genotypes and virulence, although hosts are often infected with multiple genotypes of *T. gallinae*. While this study analyzes the relationships between genotypes and pathogenicity based on epidemiological data, experimental exploration is needed to fully understand the relationships among genotypes, pathogenicity, and virulence. Further investigation by specialists in the field is necessary to elucidate these aspects.

In our study, we identified host species as a key factor influencing variations in *T. gallinae* infection rates. We observed that the Phaps genus exhibited a higher infection rate compared to Columba, potentially linked to wing length differences as discussed by Stockdale et al. (2014). Additionally, we noted variation within the same genus; for instance, *C. oenas* displayed an 83.5% positivity rate, aligning with Marx et al. (2017). Dunn et al. (2023) discovered that turtle doves genetically closer to *T. gallinae* had wings approximately 6 mm longer than those more closely related to *T. vaginalis*-like strain, suggesting a preference of *T. gallinae* for Otidiphaps. As a multi-host parasite, *T. gallinae* infects Passeriformes, Columbiformes, and Falconiformes. Our analysis focused on Columbidae without examining Passeriformes and Falconiformes. Falconiformes, predators of small birds, tend to harbor more *T. gallinae* strains, corroborating Martínez-Herrero et al. (2014). Additionally, the infectivity of *T. gallinae* varied with hosts ages, peaking at 30–180 days and lowest beyond 180 days, consistent with Jiang et al. (2016). Saikia et al. (2021) also mentioned in their article that squabs were more susceptible to and had a higher mortality rate from *T. gallinae*. Pigeons aged <30 days that were severely infected with *T. gallinae* may die, and these pigeons had not been included in the scope of epidemiological surveys. And young and adult pigeons often exhibited latent infections. This had led to the high positivity rate in young pigeons and low positivity rate in squabs, according to epidemiological surveys. Furthermore, the pH of squab oral environments was conducive to *T. gallinae* survival, making them more susceptible to *T. gallinae* infection (Martínez-Herrero et al., 2014). Parent pigeons transmit *T. gallinae* to squabs via regurgitated "pigeon milk," contributing to the infection cycle. Sex also influenced positivity rates, with insignificant differences reported by Begum et al. (2008), Mohamed (2015), and Arfin et al. (2019), possibly due to small sample sizes. Our comprehensive analysis revealed a significant sex difference, emphasizing the need for further animal regression studies to definitively assess the impact of sex on *T. gallinae* infection rates in hosts.

T. gallinae infection rates were influenced by both biological and non-biological factors, including spatial and temporal factors. Significant variations in *T. gallinae* positivity rates among continents were observed, with the lowest rate in North America and the highest in Oceania. These differences may be attributed to variations in climatic conditions and host species across continents. Further analysis revealed that temperature alone does not appear to significantly affect the prevalence of *T. gallinae*; nevertheless, precipitation levels do. A model examining the correlation between climatic conditions and *T. gallinae* positivity rates revealed that low temperatures and precipitation levels result in higher positivity rates, but the impact becomes positive when exceeding a certain threshold. High temperatures and precipitation levels may also lead to increased *T. gallinae* positivity rates. Previous studies support the notion that humid environments favor *T. gallinae* survival. Bunbury et al. (2007) identified the second stage of this pattern in their investigation in 2007. McBurney et al. (2017) confirmed in their experiment that moist environments favor the survival of *T. gallinae*. These findings enhanced our understanding of global distribution patterns of *T. gallinae*, aiding in the development of preventive measures for *T. gallinae* infections.

Time also appeared to be one of the reasons for differences in *T. gallinae* positivity rates. Between 1998 and 2003, the infection rate of *T. gallinae* in Columbidae was only 14.8%; nonetheless, between 2004 and 2009, the positivity rate skyrocketed to 61.6%. Subsequently, as management practices and rearing conditions improved and the environment changed, the positivity rate of *T. gallinae* decreased. In Guangdong Province, China, *T. gallinae* infection rates in meat pigeons have been reported since 2009, with a positivity rate of 33.9% in 2009 (Qiu et al., 2012), 36.7% in 2017 (Qiu et al., 2017), and 38.9% in 2020, and a recent publication in 2022 reported a decrease in the *T. gallinae* positivity rate to 26.6% (Cai et al., 2022). These findings were consistent with the overall global analysis results. To understand the factors leading to this rise and fall in *T. gallinae* positivity rates, investigations, such as those conducted by Alasadiy et al. (2022), have suggested that artificially reared pigeons exhibit higher positivity rates than wild pigeons, a phenomenon potentially attributed to the rearing environment. In addition, Qiu et al. (2012) found rearing environments with high management standards to have lower *T. gallinae* positivity rates, while those with poor management practices exhibited high positivity rates. These findings underscored the importance of appropriate management practices and environments in controlling *T. gallinae* infections. The fluctuations in positivity rates over time emphasized the need for ongoing surveillance and monitoring to better understand and manage this pathogen.

Although we gathered useful information from various articles and conducted comprehensive analyses, several factors that potentially affected our conclusions remain. These include host mortality, low numbers of certain bird species, unclassified hosts in the reports, and unmarked ages and sexes, all of which potentially induce biases in positivity rates. Furthermore, we regretted that some studies did not perform genotype identification, resulting in missing information for our typing analysis. In addition, climate information from some articles referred to earlier years, and finding the corresponding data for these years proved challenging. Consequently, we could only construct

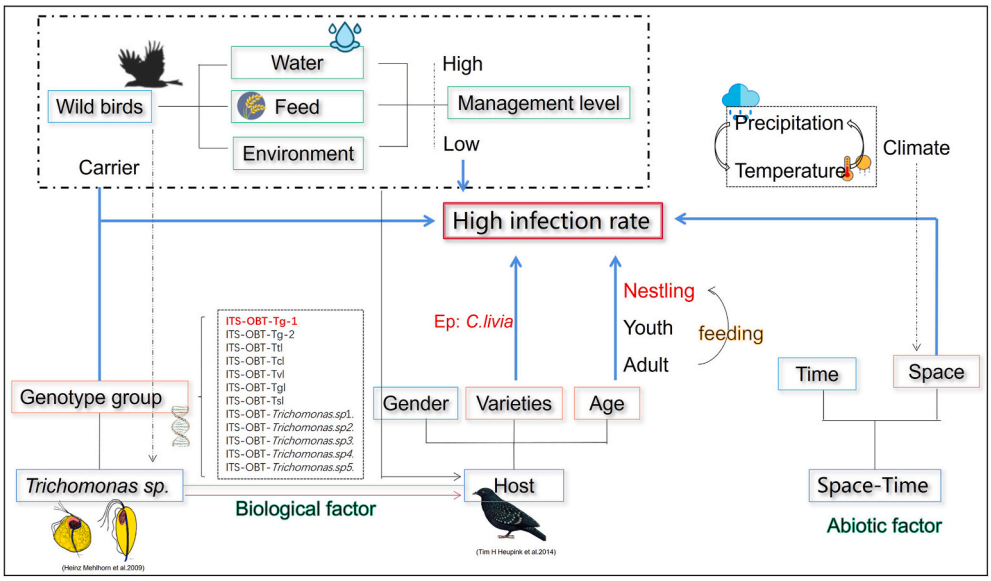

Fig. 2. Time-driven diagram of the epidemic characteristics of *T. gallinae*. The red font in the genotype box indicated *T. gallinae* genotypes with high prevalence rates. The other red font denoted highly susceptible animals, whereas the thick blue arrow indicated the factors influencing the differences in *T. gallinae* positivity rates. The content inside the orange box highlighted the main factors affecting the differences in *T. gallinae* positivity rates. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

models based on the available data for the current year, potentially leading to some degree of deviation. We regretted that these uncontrollable factors could not be mitigated, and we hope that improved methods will be developed in the future to address these issues.

5. Conclusion

To better summarize the conclusions drawn in this article, an event-driven diagram was created (Fig. 2). The current epidemic trend of *T. gallinae* is predominantly attributed to two major factor types: biological and non-biological. Biological factors include host species, age, and sex; *T. gallinae* genotype; and the unique feeding behavior between adult and nestling. The highly virulent ITS-OBT-Tg-1 genotype was widely distributed among hosts and causes various typical clinical symptoms. Nestlings and Columbidae, especially *Otidiphaps*, were important factors in the long-term spread of *T. gallinae* owing to their high susceptibility to infection. Non-biological factors, such as time, space, climate, and human-led breeding management environments, also contributed to the epidemic trend of *T. gallinae*. Domestic Columbidae, such as *C. livia*, exhibited a higher incidence rate in captive environments than in their natural habitats. High temperature and humidity could cause hosts to drink more water, and wild birds primarily transmitted the disease via contaminated water sources and food, thus limiting contamination rates per unit area. In contrast, captive environments had smaller spaces with greater host numbers per unit area, resulting in a higher risk of infection. By analyzing various aspected of the epidemiological characteristics of *T. gallinae*, we hope to effectively prevent the spread of the disease from its source, reduce economic losses, and protect species resources. This comprehensive background will also serve as a valuable resource for future research by scholars.

Authors' contributions

Y.L. and H.C. were responsible for references collection, data analysis, manuscript writing, figure and table construction, and data interpretation. D.W., S.L., N.Q., J.L., Z.Y., H.S., S.F., M.L. contributed to the supervised the project, and manuscript writing. X.L., J.H., Y.S., X.C., L. Y., D.W. and J.Z. contributed to the manuscript writing, and data interpretation. Y.G. and M.S. conceived the manuscript, supervised the project, wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

None.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The all authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2024.100918.

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