

## TRICHOMONAS GALLINAE IN MAURITIAN COLUMBIDS: IMPLICATIONS FOR AN ENDANGERED ENDEMIC

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**ABSTRACT:** Although well known as a widespread parasitic disease of columbids and birds of prey, there have been few studies of trichomonosis in populations of wild birds. In Mauritius, trichomonosis has been highlighted as a major threat to an endangered endemic, the Pink Pigeon (*Neosoenas [Columba] mayeri*). In this study, we examined the role that populations of other columbids in Mauritius might be playing as infectious reservoirs of the causal flagellate protozoan, *Trichomonas gallinae*. We screened 296 wild individuals of three columbid species (Madagascan Turtle Dove [*Streptopelia picturata*], Spotted Dove [*Streptopelia chinensis*], and Zebra Dove [*Geopelia striata*]) between September 2002 and April 2004. Prevalence varied significantly among species (ranging from 19% in *S. chinensis* to 59% in *G. striata*) and between *S. picturata* sampled from upland and coastal sites; *S. picturata* from upland sites (>500 m) were significantly less likely to be infected with *T. gallinae* than those from lowland sites (<50 m; 62% and 27% prevalence, respectively). There was no significant difference in the prevalence of *T. gallinae* at sites where Pink Pigeons were also present compared to those sampled at sites without Pink Pigeons. We show that *T. gallinae* infection prevalence is higher at sites and times of warmer temperatures and lower rainfall.

**Key words:** Conservation, introduced species, Madagascan Turtle Dove, Pink Pigeon, *Streptopelia picturata*, trichomoniasis, *Trichomonas gallinae*, trichomonosis.

### INTRODUCTION

The parasitic disease trichomonosis, caused by the protozoan flagellate *Trichomonas gallinae*, is common in columbids and birds of prey worldwide. The natural host is thought to be the Rock Pigeon (*Columba livia*) and other columbids (Stabler, 1954). Transmission is primarily direct, the parasite being passed between adults and nestlings during feeding or between adult birds during courtship behaviors. However, drinking water and food have been identified also as alternative transmission routes (Stabler, 1954; Kocan, 1969). Typical clinical signs of trichomonosis include caseous, proliferative, fibronecrotic lesions in the oropharynx and upper digestive tract which frequently lead to the death of the infected bird by starvation. Trichomonosis therefore has important commercial implications for pigeon breeding, aviculture (McKeon et al., 1997), game birds (e.g.,

Mourning Doves [*Zenaid macroura*] in the US [Stabler, 1951, 1954; Conti, 1993] and Wood Pigeons [*Columba palumbus*] in Europe [Höfle et al., 2004]), the poultry industry (Stabler, 1951, 1954), and falconry practice (Krone et al., 2005). Furthermore, the disease is increasingly recognized as a problem in the conservation management of endangered species of these avian taxa as it might pose a threat to populations into which the parasite has been introduced recently. It has been proposed, for example, that trichomonosis might have contributed to the demise of the Passenger Pigeon (*Ectopistes migratorius*) following the introduction of feral Rock Pigeons to North America (Haugen, 1952; Stabler, 1954). The parasite has also been highlighted as a possible threat to populations of native Mourning Doves in Florida (Conti and Forrester, 1981; Conti et al., 1985), Cooper's Hawks (*Accipiter cooperi*) in Arizona (Boal et al., 1998), Northern Goshawks (*Accipiter gentilis*) in

England (Cooper and Petty, 1988), and as a potential future concern for Bonelli's Eagle (*Hieraetus fasciatus*) in Spain (Real et al., 2000). The disease has been implicated recently in Greenfinch (*Carduelis chloris*) and Chaffinch (*Fringilla coelebs*) die-offs in the UK (Holmes and Duff, 2005; Lawson et al., 2006). Despite these reports, there remains a paucity of published information on *T. gallinae* in wild avian populations and detailed studies of this pathogen are clearly required to inform conservation biologists and practitioners.

In Mauritius, *T. gallinae* is highly pathogenic to the endangered Pink Pigeon (*Neosoenas [Columba] mayeri*) and has been highlighted as a threat to the species' recovery (Swinerton 2001; Stidworthy et al., 2004; Swinerton et al., 2005; Bunbury, 2006). The wild Pink Pigeon population underwent a substantial increase from less than 20 individuals in the early 1990s to over 300 birds by 2000 due to the efforts of a pioneering conservation program set up in the 1970s by the Durrell Wildlife Conservation Trust, which has been managed subsequently by the Mauritian Wildlife Foundation and the government National Parks and Conservation Service. However, the population size has remained relatively constant for the last 5 yr (Bunbury, 2006), and parasitic disease, particularly trichomonosis, is thought to be limiting population recovery by reducing reproductive success (Swinerton, 2001; Swinerton et al., 2005; Bunbury, 2006) and adult survival (Bunbury, 2006). Trichomonosis was shown recently to be the primary cause of nestling mortality in at least one Pink Pigeon subpopulation, where it was identified as the primary cause of death in a minimum of 66% of squabs in 2004 (Bunbury, 2006). The disease is also a major cause of adult mortality in all subpopulations (Stidworthy et al., 2004; Bunbury, 2006).

Although now widespread in the Pink Pigeon, the origin of *T. gallinae* infection in the species is unclear. The disease was

not seen in Pink Pigeons in the wild in Mauritius prior to 1992 and captive Pink Pigeons held in aviaries and isolated from other columbids had never showed signs of *T. gallinae* infection (C. Jones, pers. obs.). It was only subsequent to their release into the wild that trichomonosis was later recorded in these birds and their progeny. A pathogenic strain of *T. gallinae* might have been introduced to Mauritius with at least one of several species of columbids, and the *T. gallinae* reservoir is likely to include all of these. The Madagascan Turtle Dove (*Streptopelia picturata*) is very common and now considered native to the Mascarenes following the recent discovery of sub-fossil material on all three islands (Cheke, 2005), and another four species of introduced columbids are now established (i.e., occurring in free-living breeding populations) in Mauritius. The Rock Pigeon was introduced about 1715; the Spotted Dove (*Streptopelia chinensis*) about 1781; the Zebra, Peaceful, or Barred-ground Dove (*Geopelia striata*) in the 1700s (Cheke, 1987; Jones, 1996); and, most recently, the Senegal or Laughing Dove (*Streptopelia senegalensis*), which is now well-established along much of the west coast of Mauritius (C. Jones, pers. obs.). Several other species have been recorded free-living in Mauritius (Jones, 1996; C. Jones, pers. obs.) but are not yet considered established. These include the African Collared Dove (*Streptopelia roseogrisea*), the Barbary Dove (*Streptopelia risoria*), and the Diamond Dove (*Geopelia cuneata*), where the founders appear to have been released or escaped from private collections on the island.

In this paper, we explore whether wild columbids in Mauritius might be acting as potential reservoirs of *T. gallinae* by investigating *T. gallinae* infection prevalence in three of these species which directly or indirectly interact with Pink Pigeons. The Madagascan Turtle Dove frequently occurs in Pink Pigeon habitat, particularly native forest, whereas the

Spotted and Zebra Doves are more commonly found in the drier lowland secondary habitats (Jones, 1987, 1996). However, all four species show some degree of overlap in terms of habitat and food resources. We determine the prevalence of *T. gallinae* infection, using the culture method, in different species, examine geographical differences in prevalence, and analyze the effects of climatic variables on *T. gallinae* prevalence. We also investigate potential routes of inter species transmission of *T. gallinae* by testing water and supplementary food sources. The implications of our findings for the conservation management of the Pink Pigeon are discussed.

## MATERIALS AND METHODS

Sampling was carried out at six locations in Mauritius between September 2002 and April 2004. Four of these, Plaine Lievre (20°38'S, 57°45'E), Pigeon Wood (20°44'S, 57°48'E), Combo (20°47'S, 57°51'E), and Petrin (20°40'S, 57°46'E) are in the Black River Gorges National Park. Black River (20°38'S, 57°42'E) is on the west coast of Mauritius, and Ile aux Aigrettes (20°42'S, 57°73'E) is a 26 ha offshore coral islet 625 m from the southeast coast. Doves were trapped in wire cages or in field trapping aviaries, weighed in cloth bird bags using Pesola spring balances, banded with colored plastic rings, and visually examined. Ectoparasite intensity (hippoboscids flies [*Ornithoctora plicata*]) was quantified during standardized body searches. Each bird was crop-swabbed to test for the presence of *T. gallinae* with a sterile, disposable cotton swab moistened with lactated Ringer's solution. Swabs were used to inoculate individual InPouch TF™ culture kits (BioMed Diagnostics, San Jose, California, USA), which were then incubated at 38 C for 72 hr (see Bunbury et al., 2005). Birds were examined grossly for lesions of trichomonosis in their oral cavities and then were released immediately after screening. Samples were examined microscopically every 24 hr at 250× magnification to check for trichomonad activity. Samples in which no trichomonads were observed after 72 hr incubation were recorded as negative.

### Data analysis

We analyzed differences in *T. gallinae* prevalence between groups (species and sites)

with Pearson chi-square tests. We compared mean hippoboscids intensity between infected and uninfected Madagascan Turtle Doves with a Mann-Whitney *U*-test. Within-species analysis of site and climatic effects on *T. gallinae* prevalence was carried out using only data from Madagascan Turtle Doves. We obtained climate records from the Mauritius Meteorological Service and used air temperature and rainfall data from the meteorological stations closest to each turtle dove sampling location. We used simple binary and multiple logistic regression analyses to determine the relative effect of different variables on the probability of infection with *T. gallinae*. Total monthly rainfall, mean monthly temperature, and body weight were entered as factors in separate binary logistic regression analyses, and these factors, together with hippoboscids intensity, presence/absence of Pink Pigeons, and site, were factors in the multiple regression analysis. *Trichomonas gallinae* infection status was entered as the response variable. Backward deletion of variables was applied in building the model and the criterion for inclusion or exclusion was  $P < 0.05$ . Statistical analysis was carried out in SPSS v. 12.01 for Windows.

### Routes of parasite transmission

To investigate possible transmission routes of *T. gallinae* within and between Mauritian columbid species, we sampled water sources observed to have been used by at least one species of columbid at screening sites. We pipetted a few drops of water (using sterile plastic pipettes) from small, shallow sources frequently visited by birds, into individual InPouch TF™ culture kits and incubated these samples as described above. We also observed color-marked Pink Pigeons at supplementary feeding stations and collected individual grains of wheat as soon as they were dropped by birds that previously tested positive for *T. gallinae* by culture. Individual pieces of grain were collected with sterile tweezers, placed into separate culture kits, and incubated to test for the presence of *T. gallinae*.

## RESULTS

### Total prevalence and species differences

Between September 2002 and April 2004, 296 doves of three species were screened; these included 247 Madagascan Turtle Doves, 32 Spotted Doves, and 17 Zebra Doves. Overall prevalence of *T.*

TABLE 1. Number of individuals of different species of exotic columbids screened at all sites and number (%) infected with *Trichomonas gallinae*.

	Madagascan Turtle Dove		Spotted Dove		Zebra Dove	
	<i>n</i>	No. (%) infected	<i>n</i>	No. (%) infected	<i>n</i>	No. (%) infected
Plaine Lievre	16	6 (38)	8	2 (25)	4	1 (25)
Pigeon Wood	44	10 (23)	1	–	0	–
Combo	9	2 (22)	4	1 (25)	0	–
Petrin	34	9 (27)	4	0 (0)	0	–
Ile aux Aigrettes	104	64 (62)	0	–	9	7 (78)
Black River	40	24 (60)	15	3 (20)	4	2 (50)
Total	247	115 (47)	32	6 (19)	17	10 (59)

*gallinae* infection across all three species was 44.3%. We found significant differences in infection prevalence between species, which varied from 19% in Spotted Doves to 59% in Zebra Doves ( $\chi^2=10.51$ ,  $P=0.005$ ,  $df=2$ , Table 1). In addition to these species, four Senegal Doves were captured and tested in the Black River area; two were positive for *T. gallinae* infection, but these were not included in the statistical analyses.

Only one of the birds had gross lesions resembling those of trichomonosis. A Madagascan Turtle Dove had a small, whitish lesion on its soft palate. The bird was positive for *T. gallinae*, and when examined 2 mo later during a follow-up study, the lesion had disappeared.

#### Geographic differences

Our remaining analysis focuses only on the larger, and therefore statistically robust, dataset collected for the Madagascan Turtle Dove ( $n=247$  birds). Individuals sampled at Combo were excluded from the site/climate analysis because only nine birds were screened at this site and no representative climate data were available for this area. *Trichomonas gallinae* infection prevalence varied significantly across the four sampling sites ( $\chi^2=28.84$ ,  $P<0.001$ ,  $df=4$ , Table 1) with birds from Ile aux Aigrettes showing the highest infection prevalence (62.1%) and those from Pigeon Wood the lowest (23%). We divided sample sites into upland (>500 m; Plaine Lievre, Pigeon Wood, Petrin) and

lowland (<50 m; Ile aux Aigrettes, Black River) and found that *T. gallinae* infection prevalence was significantly higher in birds from lowland sites (62%) than those from upland sites (27%,  $\chi^2=27.76$ ,  $P<0.01$ ,  $df=1$ , Fig. 1). There was no significant difference in *T. gallinae* prevalence between doves sampled at sites where Pink Pigeons were also present (Plaine Lievre, Pigeon Wood, Combo, and Ile aux Aigrettes) compared to those sampled at sites without Pink Pigeons (Petrin and Black River;  $\chi^2=0.20$ ,  $P=0.66$ ,  $df=1$ ).

#### Body weight and *T. gallinae* infections and hippoboscid intensity

Mean body weight of birds infected with *T. gallinae* was significantly lower than in uninfected birds (infected:  $184.4 \pm 2.5$  g SE; uninfected:  $191.8 \pm 2.8$  g;  $t=1.99$ ,  $P=0.048$ ). Despite this difference, logistic regression indicated that body weight is a poor sole predictor of *T. gallinae* infection (Table 2). Hippoboscid intensity did not vary significantly between infected and uninfected birds (mean hippoboscid intensity of infected birds:  $0.38 \pm 0.11$  SE flies per bird; uninfected:  $0.5 \pm 0.12$ ; Mann-Whitney  $U=6903$ ,  $P=0.097$ ).

#### Effect of climate

Prevalence of *T. gallinae* infection was significantly higher at warmer (infected:  $25.3 \pm 0.25^\circ\text{C}$ ; uninfected:  $23.7 \pm 0.29^\circ\text{C}$ ; Wald=16.14,  $P<0.01$ ) and drier (infected:

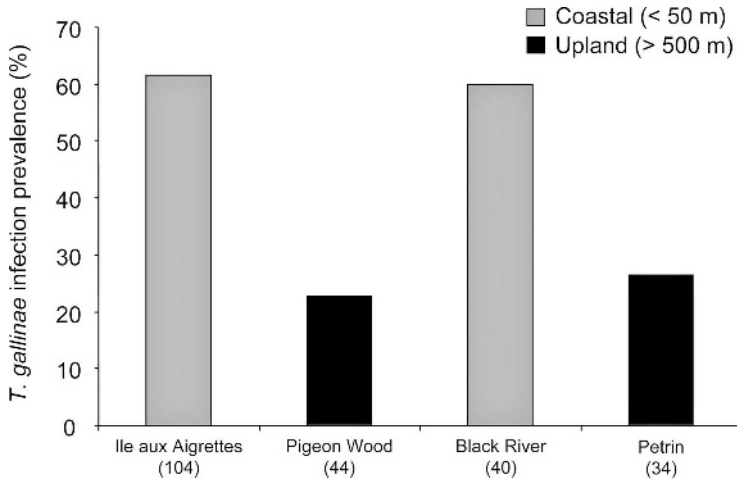


FIGURE 1. Prevalence (%) of *Trichomonas gallinae* infection in Madagascan Turtle Doves from two upland and two lowland sites in Mauritius. Pink Pigeon subpopulations are present at Ile aux Aigrettes and Pigeon Wood and absent from Black River and Petrin.

185.6 ± 14.9 mm; uninfected: 254.0 ± 16.5 mm; Wald=8.30,  $P < 0.01$ ) sites and months. Although both temperature and rainfall emerged as significant explanatory variables in simple logistic regressions, temperature appears to be a stronger predictor variable than rainfall for the observed pattern of *T. gallinae* prevalence. Temperature explains a higher percentage of the variation in the occurrence of *T. gallinae* infection than rainfall (Nagelkerke  $R^2 = 0.10$  and  $0.05$ , respectively, Table 2). In order to assess the fit of the predicted data of the model to the observed data, we presented the results

for the two climatic explanatory variables, temperature and rainfall, as fitted logistic regression curves overlaying histograms representing the observed data following Smart et al. (2004). The fitted curves depicting predicted probabilities show a better fit to the observed temperature data than to the rainfall data (Fig. 2).

After excluding the nonsignificant variables (hippoboscid intensity, Pink Pigeon presence/absence, and rainfall), the final model of the multiple logistic regression showed an interaction between temperature and body weight and a single explanatory factor, site (overall model:  $\chi^2 = 43.64$ ,

TABLE 2. Results of three separate binary logistic regression analyses for the explanatory variables body weight, temperature, and rainfall and the response variable *T. gallinae* infection prevalence. The constant represents the intercept of the model.

	Predictor variables	Estimate	SE	Wald	P	Odds ratio	95% CI of odds ratio		Nagelkerke $R^2$
							Lower	Upper	
1	Body weight	-0.009	0.005	3.86	0.05	0.99	0.98	1.00	0.02
	Constant	1.62	0.87	3.44	0.06	5.03			
2	Temperature	0.20	0.05	16.14	<0.01	1.23	1.11	1.35	0.10
	Constant	-5.04	1.26	16.04	<0.01	<0.01			
23	Rainfall	-0.002	0.001	8.30	<0.01	0.99	0.99	1.00	0.05
	Constant	0.43	0.22	4.01	0.05	1.54			

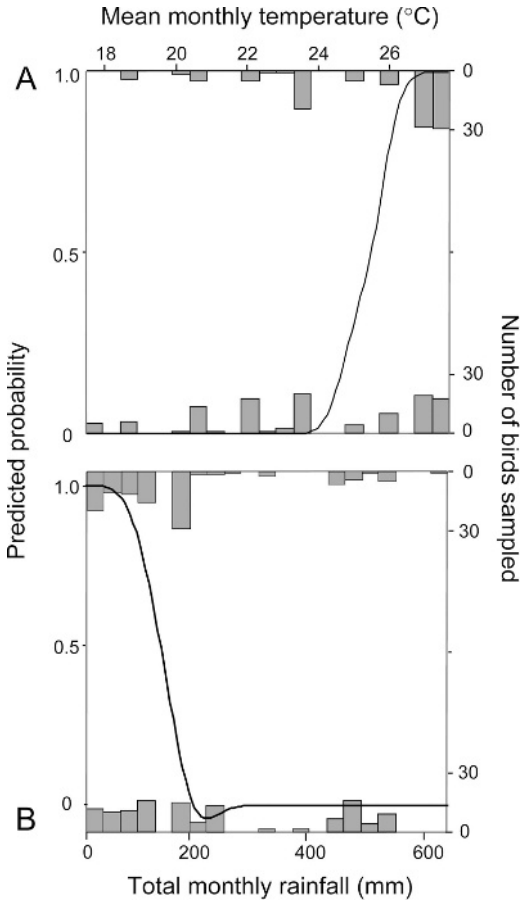


FIGURE 2. Fitted logistic regression curves showing that the probability of *Trichomonas* infection in Madagascan Turtle Doves is dependent on (A) temperature and (B) rainfall. Histograms represent the observed data and the line is the probability that a bird will be infected.

$P < 0.01$ ,  $df = 4$ ; interaction:  $\chi^2 = 14.64$ ,  $P < 0.01$ ,  $df = 1$ ). The significant interaction between temperature and body weight resulted from a contrasting weight-temperature relationship in infected and uninfected birds; uninfected birds were heavier at higher temperatures, whereas infected birds were lighter. The strength of the model's association between explanatory variables and infection prevalence was relatively high (Nagelkerke  $R^2 = 0.25$ ); thus, the model explained a considerable proportion of the *T. gallinae* infection prevalence in Madagascan Turtle Doves found in this study.

#### Routes of transmission

We sampled water from 15 different sources across the five Pink Pigeon sub-populations, including puddles, gutters, and pooled water from the roofs of buildings. We detected live trichomonads in two of these 15 samples, both of which were collected on Ile aux Aigrettes from small pools of water in the foundations of the trapping aviaries. We collected dropped grain from 15 *T. gallinae*-infected Pink Pigeons and detected no parasites in any of these samples, even after 5 days of incubation.

#### DISCUSSION

In the present study, we found an overall *T. gallinae* infection prevalence of 44.3% in three species of introduced and native Mauritian columbids. Prevalence varied significantly among species, with Zebra Doves showing the highest (59%) and Spotted Doves the lowest (19%).

These prevalences are higher than those reported in the only previous study of *T. gallinae* in the Madagascan Turtle Dove, Spotted Dove, and Zebra Dove in Mauritius which detected an overall mean prevalence of 17% in these species between 1987 and 1998 using wet-mount microscopy (Swinnerton et al., 2005). The latter detection method has since been shown to underestimate the prevalence of this parasite by over 50% when compared to culture methods. Bunbury et al. (2005) found *T. gallinae* infection prevalence to be 60% in Pink Pigeons from one site which were tested by culture, but less than half of these infected birds tested positive for *T. gallinae* by wet-mount microscopy. Our results are supportive of previous findings that the InPouch method is the most appropriate and accurate diagnostic test for *T. gallinae* under field conditions.

*Trichomonas gallinae* infection prevalence in Madagascan Turtle Doves differed significantly between sites, with birds sampled at lowland (<50 m) sites

having a higher prevalence of infection than birds sampled from upland (>500 m) locations. This difference can be explained by the significant association between temperature and rainfall with *T. gallinae* infection found in this study. Higher temperatures and lower rainfall (typical coastal weather conditions) were both associated with higher infection prevalence. Temperature was the more powerful explanatory variable, presumably because the parasite can live longer in warmer conditions. Indeed, the only water sample from which we were able to culture trichomonads was collected from Ile aux Aigrettes, which is the warmest, driest site. *Trichomonas gallinae* is incapable of encystment, so is susceptible to desiccation and therefore unable to survive for long periods of time outside the host (Stabler, 1954). Thus, at higher temperatures, and provided that external conditions are not too dry, parasites might be able to persist longer, independent of hosts, and thus have increased probability of being transmitted to a new host.

Parasite transmission via food is less likely than via a water source because the mucus medium and any *T. gallinae* protozoa on food are likely to desiccate more rapidly. This is supported by our successful culture of *T. gallinae* organisms from a known columbid water source, and the negative results of our attempts to culture the parasites from grain dropped by infected Pink Pigeons. We also found no significant effect of the presence of Pink Pigeons, and therefore supplementary feeding areas, on *T. gallinae* prevalence in doves. If drinking water sources are important routes of transmission between and within species, as suggested elsewhere (Stabler, 1954), it follows that with declining numbers and volumes of water sources, the chances of parasite transmission are likely to increase. Higher densities of birds aggregating at limited water sources could promote *T. gallinae* transmission, because parasites are more likely to be both deposited into pools via

infected hosts and then transmitted to uninfected hosts.

The predictor variables of *T. gallinae* infection probability tested here varied in their effects. Although infected birds were lighter in weight, the relationship between body weight and infection status does not indicate a clear cause and effect; light birds might be more susceptible to infection, or infection might result in reduced body weight. Because our multiple regression model described only a relatively small proportion of the variation of *T. gallinae* infection prevalence, other variables must also influence *T. gallinae* and should be investigated in order to understand the epidemiologic factors involved. These might include biotic factors such as genetic differences in *T. gallinae* strains or hosts, presence or absence of other potential *T. gallinae* host species, and presence of other pathogens in hosts or host populations that might compromise the health of individuals and their ability to tolerate *T. gallinae* infections. Abiotic factors such as other climatic variables (e.g., humidity and wind), water type, and habitat structure might also affect *Trichomonas* prevalence. For example, nestling Cooper's Hawks sampled in urban areas showed significantly higher prevalences of *T. gallinae* than those sampled in more rural areas, probably due to the higher proportion of feral Rock Pigeons in the diet of urban hawks (Boal et al., 1998).

Although pathogenicity of *T. gallinae* appeared to be very low in the Madagascan Turtle Doves, Spotted Doves, and Zebra Doves in our study, there is increasing circumstantial evidence that these infected doves are carrying strains that are pathogenic for endangered Pink Pigeons. The Pink Pigeons are supplementary-fed on wheat and, although the feeding stations are designed to exclude direct access by other avian species, other birds are attracted to these feeding sites, which might increase sharing of local water sources and thereby facilitate parasite transmission. None of the 15 grains dropped by infected birds tested positive in culture for *T.*

*gallinae* but larger sample sizes are required to determine the role that such grain around supplementary feeding areas might play in parasite transmission. However, there was also no difference in infection prevalence of *T. gallinae* in Madagascan Turtle Doves between sites with and without Pink Pigeons and their supplementary feeding stations. Two sites (Black River and Petrin) have been proposed as additional sites for Pink Pigeon reintroduction. Doves sampled at both locations tested positive for *T. gallinae* in the present study, highlighting the potential disease risk for Pink Pigeons released at these sites. Because we have shown that *T. gallinae* prevalence is correlated with temperature and rainfall, a Pink Pigeon reintroduction in the coastal Black River area is more likely to be affected by trichomonosis than an upland area. However, the proposed release site is also close to a river, providing ample watering sites for the pigeons, which might help to reduce the rate of parasite transmission. Trichomonosis has been shown to be a major cause of Pink Pigeon squab mortality in another coastal subpopulation, on Ile aux Aigrettes, (Swinerton et al., 2005; Bunbury, 2006) where fresh water is limited and increased sharing of water sources occurs. Treatment of infected free-living birds is problematic, so the focus of disease management strategies should be on monitoring and prevention rather than cure. Genetic analysis is underway to investigate strain differences in *T. gallinae* between host species and populations and also pathogenicity.

This study is the first, to our knowledge, to link the prevalence of *T. gallinae* infection with climatic factors, particularly temperature. The research might therefore influence the way *Trichomonas* is studied in birds. The study highlights the need for pathogen monitoring, not only in endangered species, but also in the neighboring common or introduced species which might share the same parasites and therefore act as sources of transmission. The work also underlines the

value of interdisciplinary research between conservation biologists, veterinarians, and wildlife disease biologists.

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