

***Trichomonas gallinae* in wintering Common Wood Pigeons *Columba palumbus* in Spain**

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We studied the prevalence and potential subclinical effects of infestation by *Trichomonas gallinae* in 91 hunter-harvested Common Wood Pigeons *Columba palumbus* from northern ($n = 30$) and southern ($n = 61$) Spain during the winter period. All animals were measured, sexed, aged, necropsied and their organs were weighed. Infestation with *T. gallinae* was diagnosed using three different methods: direct inspection for the presence of lesions, direct microscopic observation and culture. Of the sampled birds, 34.2% were positive for the presence of *T. gallinae*. Prevalence was significantly higher in adult Wood Pigeons than in the juvenile group, and prevalence was significantly lower in birds sampled from the north. No significant differences in prevalence were found between males and females. Culture was significantly more sensitive than the other methods of diagnosis. Parasitized birds were in poorer body condition, as revealed by their lower body mass and fat reserves. No significant variation could be detected in heart or spleen weight between parasitized and healthy birds. However, juvenile Wood Pigeons in which *T. gallinae* was detected had a significantly larger bursa of Fabricius. Variations in the prevalence of *T. gallinae* in Wood Pigeons could be related to migration as well as increased exposure through shared feed and water where these are artificially provided. We also discuss the potential effect of *T. gallinae* on body condition and the eventual risk for endangered predators through increased exposure to infected prey.

The flagellated protozoon *Trichomonas gallinae* is the causative agent of avian trichomoniasis, a disease affecting the upper digestive tract, primarily in columbiforms (Mehlhorn *et al.* 1992). Transmission of the parasite occurs generally when the adults feed their young but can occur through food in feeders and water (Kocan 1969). The parasite may cause severe lesions as well as subclinical infestations, depending on the pathogenicity of the strain and the immune status of the host (Mesa *et al.* 1961, Cooper & Petty 1988). Animals infested with pathogenic strains typically develop fibronectic lesions in the upper digestive tract that interfere with food and water intake and reduce body condition (Cooper & Petty 1988, Mehlhorn *et al.* 1992). Death may be due to starvation or to septicaemia from secondary bacterial infections (Mesa *et al.* 1961, Mehlhorn *et al.* 1992). Very pathogenic strains may, on occasions, cause lesions in visceral organs, and even death (Narcisi *et al.* 1991).

T. gallinae is also well known in birds of prey (Stabler 1954, Keymer 1972, Cooper & Petty 1988, Muñoz 1995, Boal *et al.* 1998, Höfle *et al.* 2000; Real *et al.* 2000), in gallinaceous (Pennycott 1998) and in psittacine birds (Baker 1986), but columbiforms are considered to be the main host and reservoir of this parasite (Mehlhorn *et al.* 1992). In columbiforms, susceptibility to infestation with *T. gallinae* and to developing lesions varies among species (Muñoz 1995). Stabler (1954) proposed that the parasite, which was introduced into North America with domestic pigeons *Columba livia* from Europe, could have been a factor in the extinction of the Passenger Pigeon *Ectopistes migratorius*. In addition, Conti (1993) warned that the introduction of the parasite into Florida with White-winged Pigeons *Zenaida asiatica* could be responsible for the decline in Mourning Dove *Zenaida macroura* populations. In birds of prey, development of the disease by nestlings was directly related to the consumption of pigeons (Cooper & Petty 1988, Boal *et al.* 1998).

Outbreaks of trichomoniasis have recently been described in Common Wood Pigeons *Columba palumbus*

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(hereafter Wood Pigeon) in the United Kingdom (Duff *et al.* 2003) and in the south of Spain (Höfle *et al.* 2004). The latter was supposedly related to enhanced transmission caused by the Wood Pigeons feeding at birdfeeders set up for pheasants and partridges on an intensive hunting estate.

Columbiforms are increasingly consumed by large accipiterine raptors in the Iberian Peninsula (Del Hoyo *et al.* 1994), as the populations of more typical prey species such as the Rabbit *Oryctolagus cuniculus* and Red-legged Partridge *Alectoris rufa* have been reduced noticeably by disease, changes in land-use and hunting pressure (Villafuerte *et al.* 1994, Gortazar *et al.* 2002). This puts nestlings of highly endangered species such as the Spanish Imperial Eagle *Aquila adalberti* or the Bonelli's Eagle *Hieraetus fasciatus* at risk from trichomoniasis, and the disease has become an important nestling mortality factor in the latter (Höfle *et al.* 2000; Real *et al.* 2000).

Studies of Mourning Doves have shown that the prevalence of infestation with *T. gallinae* may be as high as 58% in the absence of lesions (Rupiper & Harmon 1988). In addition, strains are assumed to differ in virulence and pathogenicity and although outbreaks of trichomoniasis among columbiforms are thought to be due to pathogenic strains, apathogenic strains are considered to cause protective immunity (Kocan & Knisley 1970, Sileo 1970).

Here we study the prevalence of *T. gallinae* infestations in migrating Wood Pigeons in the north of Spain during their passage through the Pyrenees, and in wintering Wood Pigeons in the south of the Iberian Peninsula, in an area with intensive gamebird management and an elevated presence of endangered birds of prey such as the Bonelli's and Imperial Eagles.

The aims of our study were (1) to evaluate potential factors that affect the prevalence of *T. gallinae* and (2) to examine the potential effect on body condition and immune capacity that *T. gallinae* may have on its host, and which may relate to disease epidemiology and to the probability of being predated and transmitting the disease to susceptible birds of prey.

MATERIALS AND METHODS

Study area

Hunter-harvested Wood Pigeons from three different hunting estates, one in the Pyrenees mountains (Etxalar; UTM 30/612800, 479000), one in south-central Spain (Peralbillo, Ciudad Real; UTM 30/413300, 4309843) and one in southern Spain (Cádiz; UTM

30/235369, 4022665) were sampled during the hunting season. The first area is a mountain ecosystem with a humid atlantic climate and a vegetation constituted mainly by European Beech *Fagus sylvatica* and Pedunculate Oak *Quercus robur*. Wood Pigeons migrate over this area because of the low altitude of the mountains and the proximity to the Cantabrian Sea. No artificial feeding or water points are available. Thus, Wood Pigeons sampled at this site are migrating birds that do not reside or stay in the sampling area.

The vegetation of the estate from south-central Spain is dominated by an open Holm Oak *Quercus ilex* woodland and interspersed dry crop fields, whereas the southern estate consists mainly of irrigation crops interspersed with mixed woodlands of Olive trees *Olea europea*, Holm Oak and Cork Oak *Quercus suber*. Rabbits are abundant in both of these latter two estates, and in the southern one there are also dense populations of Red-legged Partridges and Common Pheasants *Phasianus colchicus*. In the south-central and southern areas artificial feeding and water are available. In both areas important breeding populations of Wood Pigeons exist, and Wood Pigeon numbers increase greatly throughout the winter, as about 5 million Wood Pigeons from northern and central Europe winter in the Iberian Peninsula, especially in the described area (Purroy 1997).

Methods

We sampled a total of 91 hunter-harvested Wood Pigeons; 30 originated from Etxalar, and were captured in October; 31 originated from Peralbillo and 30 from Cádiz, in which the birds were captured in December. The northern sampling point constitutes a sample of Wood Pigeons during migration, as the method of capture only samples migrant Wood Pigeons and not resident birds. In both the southern and the south-central sampling points the Wood Pigeons captured have stayed for at least 1 month in the area, or are part of the resident population. Only juvenile birds were captured in the north, whereas both juvenile and adult birds were captured in south-central and southern Spain. Measurements of body mass, and keel, wing and tarsus length were obtained from birds hunted in south-central and southern Spain (no data were available for birds from the northern study site), and each bird was classified as juvenile or adult according to the presence of the typical white collar in adult birds (Sáenz de Buruaga *et al.* 2001).

Multivariate measures of structural size are generally preferred over univariate measures (Rising &

Somers 1989), so a Principal Components Analysis (PCA) including tarsus, wing and keel length as input variables was performed. We obtained a unique principal component (hereafter 'body size') that explained 56.2% of the variance. As an estimate of body condition we employed body mass controlled for the effect of structural size by including body size as an explanatory variable in all the analyses (García-Berthou 2001). The degree of fat deposition (a subjective index from 0 to 5) and pectoral muscle thickness, measured using a portable ultrasonic meter (Krautkramer USM 22 device) (Sears 1988), were also employed as indices of body condition of individuals. Sex was recorded for all animals and the upper digestive tract and all remaining organs were carefully inspected for the presence of lesions compatible with avian trichomoniasis. After weighing the heart, liver, spleen and bursa of Fabricius, samples of all tissues were fixed in 10% neutral buffered formalin and a second set was frozen at -20°C for further processing.

Presence of *T. gallinae* was diagnosed by direct inspection for the presence of gross lesions, direct microscopic observation of mucosal scrapings and culture as described previously (Cover *et al.* 1994, Ostrand *et al.* 1995, Höfle *et al.* 2000, 2004, Real *et al.* 2000).

Samples for culture were taken from the oropharynx, oesophagus and crop using sterile swabs and placed in sterile tubes containing 5 mL of CPLM (cysteine-peptone–liver infusion maltose) medium supplemented with 10% fetal bovine serum and 10% antibiotic solution (Sigma-Aldrich Co., St Louis, MO, USA), incubated at 36.5°C and inspected daily for 7 days for the presence of flagellated protozoa (Höfle *et al.* 2004). For direct examination a sterile cotton wool swab was moistened with saline solution, rubbed against the oral and crop mucosa, extended in a drop of saline at room temperature and examined via phase contrast microscopy at $100\times$ and $400\times$ magnification (Mehlhorn *et al.* 1992). Flagellated protozoa were identified as *T. gallinae* by morphology and polymerase chain reaction (PCR) as described in Höfle *et al.* (2004). All samples were taken less than 8 h after the bird had been shot to make sure that *Trichomonas* organisms were still alive (Erwin *et al.* 2000).

Statistical analysis

We used homogeneity tests such as χ^2 and Fisher's exact test to test against the null hypothesis of absence

of differences in *Trichomonas* prevalence in relation to sex, age or origin of the birds. We also used these tests to compare between the diagnostic techniques employed. General Linear Models (GLMs) (Crawley 1993) were employed to analyse the relationship between infestation by *T. gallinae* and splanchnometry and two of the body condition indices (body mass and pectoral muscle thickness). The third index of body condition (degree of fat deposition scored from 0 to 5 on a subjective scale) was analysed by means of the Mann–Whitney *U*-test. We employed SPSS 10.0.6 software (SPSS Inc., 1999). The use of GLMs allowed us simultaneously to assess the effect of several categorical and continuous explanatory variables and their interactions to determine which one best explained the original deviance of the data set. We employed raw data (except in the case of spleen mass, which was log-transformed for analysis) and a backward stepwise procedure.

Owing to the large number of categorical explanatory variables that could be included in the model (sex, age, hunting estate, presence of *T. gallinae*) and the limited sample size for which a complete data set was available, not all possible combinations of levels could be fulfilled. Specifically, it was not possible to include hunting estate (south-central or southern Spain), age (adult or juvenile) and presence of *T. gallinae* (detected vs. undetected) together in the same model. To solve the dichotomy between including age or estate, we decided not to include hunting estate in the analysis. This seems to be the best option as there were significant differences in prevalence of *T. gallinae* between juveniles and adults (see Results) but not between the south-central and southern estates. In addition, further analysis suggested that most of the possible variation between hunting estates could be attributed to the unbalanced proportion of juvenile birds for which a complete data set was obtained in the south-central estate (21 juveniles vs. one adult) in comparison with the southern estate (five juveniles vs. ten adults); thus, it seems more appropriate to control for the effect of age than for the effect of the hunting estate. Furthermore, wintering Wood Pigeons are extremely mobile and birds could have been moving between hunting estates, even though they are 300 km apart.

To summarize, GLMs in which *T. gallinae* infestation (presence vs. absence in culture), age, sex, body size and all two-way interactions were introduced as explanatory variables were performed for body mass, pectoral muscle thickness, and heart and \log_{10} -spleen mass. As the bursa of Fabricius is only present in

juvenile birds the GLM for bursa mass as a dependent variable (including the same explanatory variables) was performed only for this age group.

RESULTS

Prevalence of infestation

The prevalence of *T. gallinae* among the Wood Pigeons collected was 34.21%. No difference was found in the prevalence of infestation between Wood Pigeons from south-central Spain and those from southern Spain (41.94 vs. 46.67%; $\chi^2 = 0.09$, $P > 0.05$). Juvenile Wood Pigeons from the southern and south-central estates had a significantly lower prevalence of *T. gallinae* than did adult birds ($\chi^2 = 5.03$, $P < 0.03$). By contrast, the prevalence of *T. gallinae* in juveniles from the southern and south-central estates was significantly higher than it was in the northern birds that comprised only juveniles ($\chi^2 = 3.74$, $P = 0.053$) (Fig. 1).

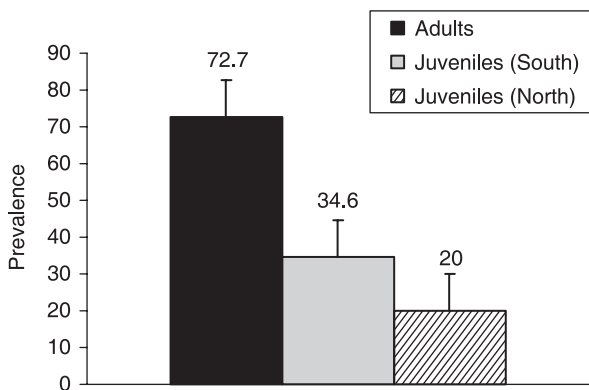


Figure 1. Differences in prevalence of *Trichomonas gallinae* between age class and origin of Wood Pigeons.

The prevalence was similar in males (36.36%) and females (53.33%) ($\chi^2 = 0.87$, $P > 0.05$).

From the total of culture-positive Wood Pigeons only 2.2% had macroscopic lesions compatible with trichomoniasis and in only 8.7% was *T. gallinae* observed on direct microscopic examination. By contrast, all lesion- or directly positive animals yielded positive cultures. Thus, culture was significantly more sensitive than inspection for lesions ($\chi^2 = 25.67$, $P < 0.001$) or direct microscopy ($\chi^2 = 4.320$, $P < 0.001$), and all or most of the subclinically infected birds would have been missed using methods other than culture.

Presence of *T. gallinae*, body condition and splanchnometry

Body mass was significantly related to age (adults being heavier than juveniles) and body size (Table 1). There was also a marginally significant interaction between body size and age, as the relationship between body size and body mass tended to be slightly stronger in adults ($r = 0.71$, $P < 0.001$) than in juveniles ($r = 0.57$, $P < 0.01$), although the slopes did not differ significantly (parallelism test: $F_{1,33} = 2.96$, $P = 0.09$). After controlling for the effects of age and body size, uninfested birds tended to be heavier than those infested by *T. gallinae* (Table 1, Fig. 2). Of the other two indices of body condition employed, pectoral muscle thickness was neither influenced by *T. gallinae* infestation nor by any of the other variables analysed (Table 1). By contrast, fat deposition index was lower for infected than for uninfested birds, a difference that was marginally significant (Mann–Whitney U -test: $Z = 1.98$, $P = 0.056$), whereas no significant differences were found between sexes ($Z = 0.07$, $P = 0.94$) or age classes ($Z = 1.22$, $P = 0.22$).

Table 1. Significant explanatory variables and interactions resulting from stepwise backward GLM. Body size, age, *Trichomonas gallinae* infestation, sex and all two-way interactions were introduced in the initial models as explanatory variables.

Dependent variable	Significant explanatory variables and interactions	F	P
Body mass	Body size	28.7	< 0.001
	Age	6.73	< 0.05
	<i>T. gallinae</i> infestation	7.66	< 0.01
	Age × Body size	4.09	0.051
Heart mass	Body size	12.6	< 0.01
Log ₁₀ spleen mass	None	–	–
Pectoral muscle thickness	None	–	–
Bursa of Fabricius mass	<i>T. gallinae</i> infestation	7.51	< 0.05

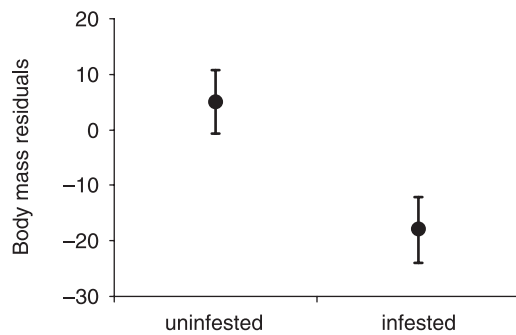


Figure 2. Residuals of body mass in birds infested or not infested by *Trichomonas gallinae* after correction for body size, age and body size–age interaction. Results are shown as mean \pm se.

Regarding splanchnometry, although heart mass was positively correlated with body size (Table 1), we found no significant predictor of the variation in spleen mass. By contrast, the size of the bursa of Fabricius was related significantly to the rate of infestation by *T. gallinae* (Table 1, Fig. 3): infested juveniles had larger bursae than uninfested juveniles.

DISCUSSION

The presence of *T. gallinae* in the upper digestive tract of free-living Wood Pigeons appears only to have been described on three occasions. One was a large-scale study in northern Spain among different host species (Muñoz 1995). The other two described outbreaks of trichomoniasis with severe lesions and high mortality among Wood Pigeons in southern Spain (Höfle *et al.* 2004) and in the south of the UK (Duff *et al.* 2003). The prevalence of *T. gallinae* among Wood Pigeons is similar to the prevalence described

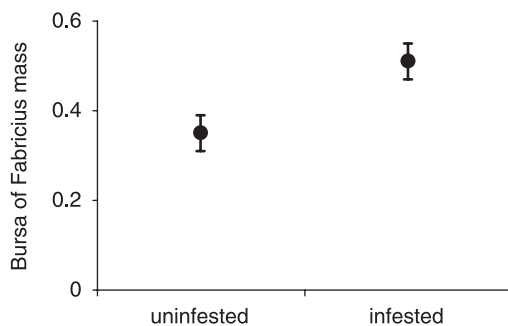


Figure 3. Weight of the bursa of Fabricius in juvenile Wood Pigeons in the presence and absence of *Trichomonas gallinae*. Results are shown as mean \pm se.

in some other free-living columbiforms, such as the European Turtle Dove *Streptopelia turtur* with a prevalence of 50% (Muñoz 1995), but in contrast to the high prevalence (92%) of the parasite observed in free-living Rock Pigeons *Columba livia* (Muñoz 1995).

A similar distribution of the parasite has been observed by different authors in American columbiforms with a prevalence of 100% in White-winged Pigeon and of 50% in the Inca Dove *Columbina inca* and the Mourning Dove (Stabler 1961). In most studies of Rock Pigeons juvenile birds are more frequently found to be parasitized by *T. gallinae* than are adults (Muñoz 1995, Ostrand *et al.* 1995), a situation that contrasts with our results for Wood Pigeons, which show a higher prevalence of the parasite among adult birds. A possible cause of this difference could be the aggregation of Wood Pigeons at game-bird feeders and water points in the south of Spain and transmission of the parasite via the grain and water (Kocan 1969). Shared food and aggregation have previously been described to increase the risk of infection of other diseases such as bovine tuberculosis in deer (Miller *et al.* 2003), and could also explain the differences in prevalence of *T. gallinae* between the juvenile Wood Pigeons in northern and southern Spain. In addition, stress from strenuous exercise and social stress from crowding during migration have been shown to render birds more susceptible to infestation as well as to reactivation of latent disease (Råberg *et al.* 1998, Gylfe *et al.* 2000). Both enhanced susceptibility immediately after migration and increased exposure to the parasite could thus explain the higher infestation rates of juvenile Wood Pigeons in the southern wintering areas in contrast to northern Spain. In fact, all of the Wood Pigeons that winter in Spain migrate through the area of the northern sampling point (Purroy 1997). As we cannot determine the sites of origin of the sampled birds, the results from the northern sampling point may reflect the prevalence of trichomoniasis in the locations of origin. The Wood Pigeons sampled in the southern areas had stayed in the region for 1–2 months at sampling. Thus, we cannot exclude the possibility that the latitudinal pattern found here reflects differences in time that are mediated partly by the differences of prevalence in the locations of origin of each individual that may have affected their condition, rather than their local exposure. Our results may also mean that prevalence of *T. gallinae* in Wood Pigeons in the absence of artificial feeding might have been lower, and underline the potential of feeding in the

promotion of disease outbreaks, as has been described for trichomoniasis, avian tuberculosis and salmonellosis (Kirkwood 1998, Höfle *et al.* 2004, Millán *et al.* 2004).

Several authors have found a sex-related difference in susceptibility to parasitism and diseases, with males being generally more susceptible to negative effects of parasitization (Alexander & Stimson 1988, Møller *et al.* 1998, Blanco *et al.* 2001). We could not confirm this finding in our study, possibly because we collected our study birds outside the breeding season, in contrast to, for example, Blanco *et al.* (2001), and after migration, which supposes a similar cost for both sexes.

In our study, culture was more sensitive for detection of *T. gallinae* than was direct microscopy, as has been stated by other authors (Diamond 1954, Kocan & Knisley 1970). Some authors have nevertheless found direct examination to be more effective for the detection of the parasite (Schmid *et al.* 1989, Muñoz 1995).

The low prevalence of trichomoniasis lesions among the birds positive for *T. gallinae* is consistent with the results of other studies on *T. gallinae* and trichomoniasis (Diamond 1954, Kocan & Knisley 1970, Muñoz 1995). Subclinical infestations are generally considered to be due to apathogenic strains of *T. gallinae*, although host immune status has also been reported to influence the development of clinical disease (Mesa *et al.* 1961, Kocan & Knisley 1970). However none of these authors has investigated subclinical effects that the parasite may have on its host. The observed reduction in body mass and fat deposition in *T. gallinae*-positive Wood Pigeons suggests a reduction in the ingestion of food even in the absence of trichomoniasis lesions, with which lack of ingesta and loss of condition are generally described. It could also indicate a higher energy expenditure owing to the removal of resources by the parasite and the cost of the corresponding immune response (Mehlhorn *et al.* 1992; Lochmiller & Deereberg 2000, Ots *et al.* 2001). This loss of body condition could render these animals more susceptible to predation or to other disease agents. Susceptible predators, such as the Spanish Imperial Eagle or the Bonelli's Eagle, may in consequence become exposed to the parasite. This exposure could be an important problem for raptor conservation, as trichomoniasis is one of the most important causes of nestling mortality in these endangered species (Höfle *et al.* 2000, Real *et al.* 2000). Other authors have previously reported a relationship between parasite burdens and susceptibility to predation

(Hudson *et al.* 1992, Millán *et al.* 2002). Joly and Messier (2004), in a study on *Echinococcus granulosus* in moose and their potential role in wolf predation, propose that the parasite may increase the vulnerability of its host to predation in order to ensure transmission. Interestingly, we observed that infected juveniles also had a larger bursa of Fabricius, which could be considered to be an indicator of activation of the immune defences against a current infestation (Glick 1983). Mounting an immune response is a stressful and costly activity for the organism (Råberg *et al.* 1998, Lochmiller & Deereberg 2000, Ots *et al.* 2001), which again may weaken the affected individual, rendering it even more susceptible to predation or to other agents.

Both loss of body condition and an increase in bursa size may, however, be related to other underlying causes that could in turn have predisposed the Wood Pigeons to infestation by *T. gallinae*. Bursa size, in particular, may be affected by various infectious diseases such as circovirus infections that have also been shown to increase the susceptibility of affected birds to secondary diseases such as aspergillosis or trichomoniasis (Phalen *et al.* 1991, Todd *et al.* 2001). Nevertheless, bursa size has also been shown to increase with intense parasitization as part of the immune response of the host (Keymer 1982, Glick 1983). In our case, no lesions suggestive of viral or bacterial infestations, *Chlamydia* or mycoplasma were found upon histological examination and burdens of other parasites were generally low. Thus, although detection of subclinical infections with other pathogens is pending, we hypothesize that the increase in bursa size could be related to the presence of *T. gallinae* itself or to the increased contact with other potential pathogens resulting from the breakdown of the mucosal barrier in the upper digestive tract by *T. gallinae* (Stabler 1954).

In conclusion, we found a lower prevalence of *T. gallinae* in Wood Pigeons than that which has been described for Rock Pigeons (Muñoz 1995). Although few of the infected Wood Pigeons had typical trichomoniasis lesions, subclinical effects of the infestation on the immune system and body condition were present. This could have an effect on the population ecology of free-living Wood Pigeons, exposing them to predation or other diseases. In addition, this greater susceptibility to predation of infected individuals potentially puts certain birds of prey and their nestlings at risk of exposure to the parasite. Our results suggest a possible relationship between migration, artificial feeding and the prevalence of *T. gallinae*.

This adds information as to the role of migratory birds in the transportation and spread of certain pathogens and illustrates the potential negative effects that certain management measures may have for the conservation of wildlife even if other species are targeted. Nevertheless, more research would be required to elucidate these hypotheses.

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