BOOK: *Wild Birds: Behavior, Classification and Diseases*

REVIEW CHAPTER. Tentative Title: Oropharyngeal trichomonads in wild birds.

***AUTHORS**: María Teresa Gómez-Muñoz, DVM, PhD, María del Carmen Martínez-Herrero, DVM, PhD, José Sansano-Maestre, DVM, PhD, María Magdalena Garijo-Toledo, DVM, PhD*

María Teresa Gómez Muñoz. Universidad Complutense de Madrid. Facultad de Veterinaria. Departamento de Sanidad Animal. Madrid, Spain.

e-mail: mariateg@ucm.es

María del Carmen Martínez-Herrero. Universidad CEU Cardenal Herrera. Instituto de Ciencias Biomédicas. Facultad de Veterinaria. Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos. Valencia, Spain.

e-mail: maria.martinez1@uchceu.es

José Sansano-Maestre. Universidad Católica de Valencia. Facultad de Veterinaria y Ciencias Experimentales. Departamento de Producción Animal y Salud Pública. Valencia, Spain.

e-mail: jose.sansano@ucv.es

María Magdalena Garijo Toledo. Universidad CEU Cardenal Herrera. Instituto de Ciencias Biomédicas. Facultad de Veterinaria. Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos. Valencia, Spain.

e-mail: marilena@uchceu.es

Oropharyngeal trichomonads in wild birds

María Teresa Gómez Muñoz

Departamento de Sanidad Animal, Universidad Complutense de Madrid, Spain

María del Carmen Martínez-Herrero

Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos. Universidad CEU Cardenal Herrera Valencia, Spain.

José Sansano-Maestre

Departamento de Producción Animal y Salud Pública. Universidad Católica de Valencia. Facultad de Veterinaria y Ciencias Experimentales. Valencia, Spain

María Magdalena Garijo Toledo

Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos. Universidad CEU Cardenal Herrera. Instituto de Ciencias Biomédicas. Facultad de Veterinaria. Valencia, Spain

Corresponding Author: mariateg@ucm.es

ABSTRACT

Oropharyngeal trichomonosis is a disease that affects wild and domestic birds. Some studies carried out in wildlife recovery centers pointed it out as the main cause of entrance of birds of prey due to infectious diseases. It causes small nodules and ulcers in the crop and other locations of the upper digestive system of the animals. These small lesions can coalesce and form large granulomas, which can provoke the death of the animal by starvation. *Trichomonas gallinae* is considered the etiological agent of the disease, a flagellated protozoon that is frequently found in the oral cavity of columbiformes, which are considered the main reservoir of the parasite. However, in the last decade, a great progress in the molecular characterization of this and other protozoa has been reached, and the number of genetic variants and even new species within the trichomonads that inhabit the avian oropharynx has expanded. In this review, we outline the latest descriptions of these parasites and their host spectrum; more than 10 genetic variants or new species are included. Although trichomonosis has been described in several groups of birds, the higher impact is usually found on Accipitriformes, Falconiformes and Strigiformes due to their predation or scavenging habits. Psittaciformes and Passeriformes also show clinical signs of the disease, and recently, several epidemic episodes of trichomonosis in fringilids were described across Europe and North America. In addition, chicks of endangered species like the Bonelli´s eagle (*Aquila fasciata*) are frequently affected by the parasite, as several studies carried out in Spain and nearby countries have proved. In this chapter, we summarize the most important features of the disease, including the biology, the diagnosis and treatment options. Additionally, we will describe the recent scientific advances in the pathology, epidemiology and control of the disease.

1. AVIAN OROPHARYNGEAL TRICHOMONOSIS

Avian oropharyngeal trichomonosis is a parasitic disease that has been known for centuries. Although it has been described mainly in birds, there are some references about the presence of the parasitic process affecting even dinosaurs. First documented descriptions were found in falconry books, where the disease is explained as the appearance of typical lesions on birds of prey. For example, there is a book on falconry called "The hunting of birds", from the 14th century, and the London's Turbervile book of 1575 in which the typical lesions are described.

At first, the etiological agent was named *Cercomonas gallinae* (Rivolta, 1878). Later, in the year 1938, its taxonomic classification was reviewed by Dr. Robert M. Stabler, from the University of Colorado. He is one of the authors who has contributed most to knowledge about avian trichomonosis through numerous scientific publications.

Since then, other scientists, especially in the United States, have been responsible for studying different aspects related with pathogen mechanisms, morphology, immunology, biology, and protozoan treatment. Some of the scientists to be highlighted are: Dr. Richard M. Kocan, from the US Bureau of Sport Fisheries and Wildlife, Patuxent wildlife research center, Laurel, Maryland; Dr. Bronislaw M. Honigberg, from the University of Massachusetts; Dr. Norman D. Levine from the University of Illinois and Dr. Lionel G. Warren, from Rice University, Houston, Texas, among others.

Trichomonosis is one of the most relevant infectious diseases among wild birds, both from urban (Boal *et al*., 1998; Stenkal *et al*., 2013) and rural environments. Raptors are the most frequently reported with the disease in the studies carried out. Wendell *et al*., in 2002, analyzed the causes of admission to the Veterinary Hospital of Colorado University (USA) in a retrospective study from 1995 to 1998. Trichomonosis accounted for 44% among the infectious diseases in raptors and it was the second cause of admission in importance after trauma. In a different study carried out in Spain, the authors found the same percentage of trichomonosis among the infectious diseases in raptors, being Strigiformes and Falconiformes the most affected (Molina-López *et al*., 2011). Remarkably, the disease has been considered emergent in passeriformes since 2005 (Pennycot *et al*., 2005) and it has spread out through Europe and America, being a cause of population decline.

2. THE AGENT: TAXONOMY, MORPHOLOGY, LIFE CYCLE AND TRANSMISSION

Traditionally, the etiological agent of avian oropharyngeal trichomonosis was considered *T. gallinae*. However, in the last decade several genetic variants or new species have been described. In this section, we describe *T. gallinae*, since other species and variants will be discussed in a different section.

2.1. Taxonomy

Trichomonas gallinae (Rivolta, 1878) belongs to the family Trichomonadidae, order Trichomonadida, class Trichomonadea, phylum Parabasalia (Brugerolle and Lee, 2000; Cepicka *et al.*, 2010):

Empire Biota
Domain Eukaryotes (Chatton, 1925)
Phylum Parabasalia (Honigberg, 1973)
Class Trichomonadea (Cepicka, 2010)
Order Trichomonadida (Kirby, 1947)
Family Trichomonadidae (Chalmers and Pekkola, 1918)
Subfamily Trichomonadinae (Chalmers and Pekkola, 1918)
Genus *Trichomonas* (Donné, 1836)
Species *Trichomonas gallinae* (Rivolta, 1878)

All trichomonads are unicellular flagellated protists classified in the supergroup Excavata, which includes not only parasitic organisms, but also free-living and commensal ones (Sleigh, 1991). The species grouped in the order Trichomonadida are anaerobic and present a complex cytoskeleton, three to five flagella at the apical pole, one recurrent flagellum, axostyle, costa and pelta (Mehlhorn *et al.*, 2009).

T. gallinae is classified in the subfamily Trichomonadinae, one of the two that represent the family Trichomonadidae. Other parasites of human and veterinary importance, like *Trichomonas vaginalis* or *Tetratrichomonas gallinarum* are also included in this subfamily. As these protozoa lack mitochondria, they have an alternative route for ATP synthesis that is fulfilled by hydrogenosomes, cytosolic organelles that do not require an oxygen influx.

The genus name, *Trichomonas*, derives from the Greek word "thrix-" meaning hair and "-monas" that indicates a single unit, regarding their one cell structure.

2.2. Morphology

The parasitic stage of *T. gallinae* is called “trophozoite” (from the Greek word "troph-" meaning feed and "–zoite", from "–zoon", animal). It has an average cell size of 7 to 11 µm and its morphology can vary from ovoid to pyriform or spherical. It has a great elasticity that allows frequent transitions into these different forms (Borror, 1988; Mehlhorn *et al.*, 2009; Martínez-Díaz *et al.*, 2015) (figure 1).

The trophozoite presents five flagella arising from the periflagellar canal, which is located at the apical pole of the cell. Four of them, the anterior flagella, measure from 11 to 18 µm. The fifth one,

called the recurrent flagellum, is attached to the cell membrane in all its extension. This flagellum accompanies the undulating membrane, which measures between 2/3 and 3/4 of the cell's length. Basal bodies are located at the origin of each flagellum, and present a typical structure of a central centriole and external microtubule triplets (Mehlhorn *et al.*, 2009).

It presents an oval nucleus whose mean measures are 2.5-3.4 µm length and 2.2 µm width and is found next to the basal bodies of the flagella (Mehlhorn *et al.*, 2009; Martínez-Díaz *et al.*, 2015). Near the proximal pole and close to the nucleus, a curved microtubule complex called the pelta is located (Honigberg and Brugerolle, 1990; Cepicka *et al.*, 2010). The costa is another long dimension cytoskeleton structure, located below the undulating membrane, with filaments arranged in a herringbone pattern.

Finally, the axostyle is a characteristic support structure with its final segment extending from the posterior pole of the cell. It is composed by a single row of microtubules that extend throughout the central axis of the trophozoite, starting from the nucleus up to the end of the cell and extending beyond (Stabler, 1941).

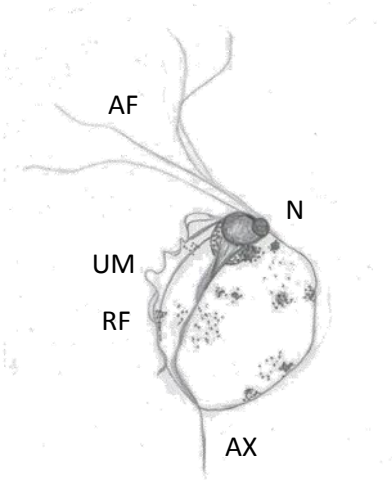

Figure 1. Schematic representation of a *T. gallinae* trophozoite. AF: anterior flagella, RF: recurrent flagellum, UM: undulating membrane, N: nucleus, AX: axostyle. Original drawing by Mónica Caridad Benito Torrecilla.

For the species *T. gallinae*, the development of a cystic wall to generate resistance forms has not been described. However, electron microscopy studies have revealed a morphotype called pseudocyst. Its morphology can be oval or spherical and has no flagella or undulating membrane. This form appears in *in vitro* conditions, and is related to stressful situations, such as an inadequate temperature. Pseudocysts have been found in intestinal trichomonads of reptiles, rodents, birds and amphibians (Mattern *et al.*, 1973; Stachan *et al.*, 1984; Friedhoff *et al.*, 1991). In addition, their formation is documented in parasitic species of the urogenital tract of bovids, such as *Tritrichomonas foetus* and

humans, such as *T. vaginalis*. These resistant forms adhere to the host tissue at a higher rate than the trophozoite stage, which indicates that they could have an important effect on the transmission and the infective capacity of the parasite (Mariante *et al.*, 2004). In addition, their transition to the trophozoite stage has been documented, being also competent to multiply (Granger *et al.*, 2000; Pereira-Neves *et al.*, 2003; Castro *et al.*, 2016).

Analysis through electronic microscopy has also allowed for a more precise study of the morphology of the trophozoite (figure 2). It has a complex cytoskeleton composed of four types of kinetosome rootlets involved in the trophozoite morphology, including the structures of the costa, pelta and parabasal filaments. In addition, some filamentous structures have been described in cytoplasmic granules associated with the axostyle and costa.

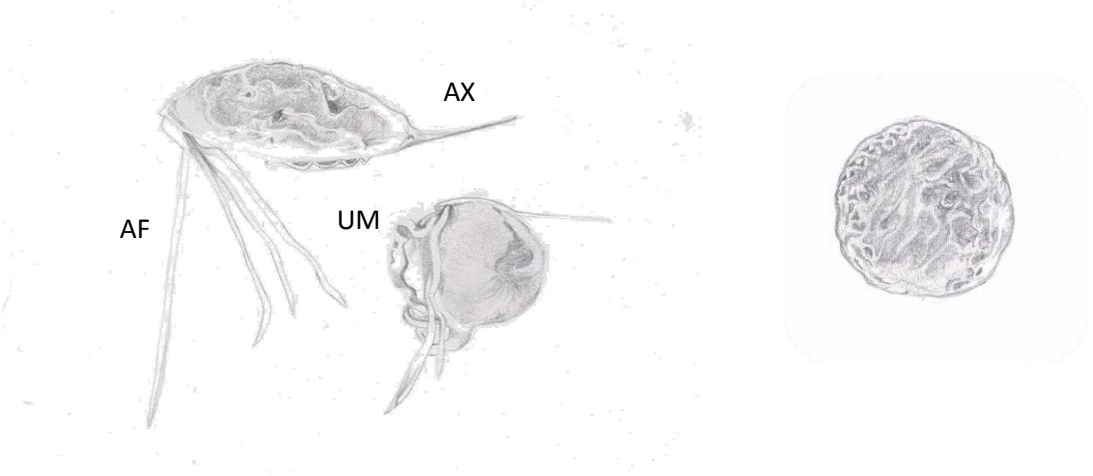

Figure 2. Trophozoites (left) and pseudocyst stage (right) of *T. gallinae*. AX: axostyle, AF: anterior flagella, UM: undulating membrane. Original drawing by Mónica Caridad Benito Torrecilla, adapted from Tasca and De Carli (2003).

2.3. Life cycle and transmission

T. gallinae has a direct life cycle, with trophozoites multiplying by longitudinal binary fission, one cell will divide to form two new ones (Stabler, 1941). Their preferred location is the mucosa of the upper digestive tract, from the oropharyngeal cavity and crop to the proximal esophagus. Nevertheless, affection of internal organs such as liver, lungs, pericardium, air sacs and pancreas, has also been reported in certain strains of the parasite. Indeed, some strains target particular tissues, namely, Jones' Barn and Eiberg strains are hepatotrophic, while Mirza strain is cephalotrophic (head sinuses, orbital regions, brain and neck) (Stabler and Engley, 1946; Jaquette, 1950; Pérez-Mesa *et al.*, 1961; Kocan and Herman, 1971; Narcisi *et al.*, 1991). However, the Jones' Barn strain primary showed tropism the lungs instead of the liver of mourning doves (*Zenaida macroura*) and rock pigeons (*Columba livia*) in

experimental infections. Non pathogenic strains, like Lahore and Stabler-gallinae (SG), have also been described (Honigberg *et al.*, 1964; Kocan, 1969a; Nadler and Honigberg, 1988).

The primary route of transmission is by direct contact with the saliva of an infected bird, although contaminated food and water also act as sources of infection (Kocan, 1969b; Kietzmann, 1990; Bunbury *et al.*, 2007). Indeed, experimental transmission of the parasite through the ingestion of contaminated food has been proved in columbiformes. Recent studies have showed that bird baths may act as potential vehicles with viable trophozoites for at least 16 hours (Purple and Gerhold, 2015; Purple *et al.*, 2015).

Contaminated carcasses also remain infective for at least 48 hours after death, acting as a reservoir for ornitophagous and scavenging birds (Erwin *et al.*, 2000). Moist grains are also able to maintain the parasite's viability for at least five hours (Kocan, 1969b).

Regarding the number of trophozoites needed to initiate the infection, it seems that only one trophozoite of the Jones' Barn strain is enough to develop the disease and cause death in pigeons in less than 15 days post-infection (Stabler and Kihara, 1954).

3. NEW SPECIES AND VARIANTS OF OROPHARYNGEAL TRICHOMONADS IN BIRDS

3.1. New species: *Trichomonas stableri*

3.1.1. *Introduction*

In 2011-2012 a new species was described in USA: *Trichomonas stableri* (Girard *et al.*, 2014a). Named in honor of Professor Robert M. Stabler (1904-1985), who was one of the great pioneers of avian trichomonosis research. This new species was isolated from adults of Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*) in California. According to the authors, the pathogenic character of the protozoan was equal to *T. gallinae*, as lesions were located in the oropharyngeal cavity, esophagus and lungs, with healthy carriers found as well. Coinfections with *T. gallinae* were also detected.

Biological factors related to the life cycle of the birds, increased their susceptibility to disease. The population studied in California performs a migration into Alaska and was subjected to game pressure, being recognized in decline since 1966 (Keppie and Braun, 2000). *T. stableri* was identified during mortality events that occurred in winter and early spring migration and in one breeding bird without clinical signs.

3.1.2. *Host spectrum*

Up to date, this species has only been reported on Pacific Coast band-tailed pigeons.

3.1.3. *Morphology*

T. stableri has four anterior flagella with the common external structures that characterize *T. gallinae*: axostyle projection in the caudal pole of the cell and a recurrent flagellum that forms the undulating membrane. Two morphotypes were described: the slender and the rounded form (figure 3). Although *T. gallinae* trophozoites have an inconstant shape, with a particular grade of pleomorphism, are generally defined with a pyriform, ovoid or spherical form (Stabler 1941; Tasca and De Carli, 2003; Mehlhorn *et al.*, 2009; Girard *et al.*, 2014a; Martínez-Díaz *et al.*, 2015).

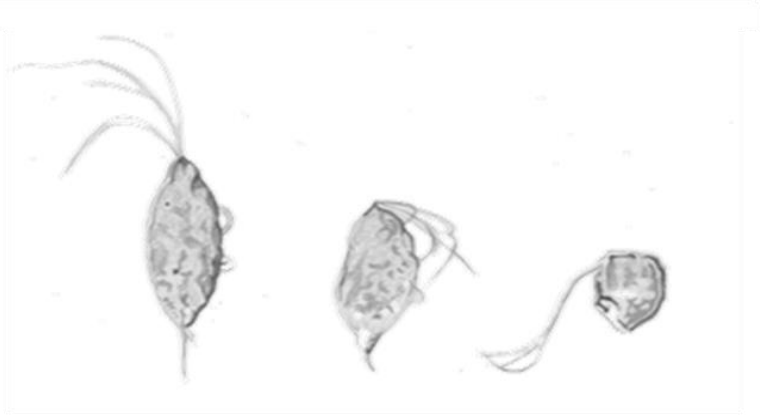

Figure 3. From left to right: *T. gallinae*, slender form of *T. stableri* and rounded form of *T. stableri*. Original drawing by Mónica Caridad Benito Torrecilla, adapted from Girard *et al.*, 2014a.

3.1.4. *Phylogenetic analysis*

Authors reported that *T. stableri* isolates had 92.1-93.4% nucleotide similarity with *T. gallinae* strains for the ITS1/5.8S/ITS2 region, while 99.6% was obtained in comparison with *T. vaginalis* strains (Girard *et al.*, 2014a).

3.1.5. *Co-existence with T. gallinae*

The band-tailed pigeon population of California, USA, was carefully studied to unravel the epidemic pattern of avian trichomonosis. Findings indicated that during non-epidemic periods, birds harbored mainly *T. gallinae* Fe-hydrogenase subtype A2, whereas *T. gallinae* Fe-hydrogenase subtype A1, *T. stableri* and *Tritrichomonas blagburni* n. sp.-like were found in lower abundance. In contrast, during the winter or early spring migration, periods where birds suffered from higher stress levels and outbreaks occurred, *T. gallinae* Fe-hydrogenase subtype A2 and *T. stableri* were isolated (Girard *et al.*, 2014b). Infection was detected in 96% of the birds affected during mortality events of trichomonosis. The epidemiology of the disease was similar to the outbreak of a wintering woodpigeon population reported from Spain (Villanúa *et al.*, 2006).

This parasitic disease was pointed out as another key factor to monitor during the study of the population dynamics, due to their steadily decline. Additionally, the detection of the causative agent of the European finch trichomonosis epidemic (*T. gallinae* Fe-hydrogenase subtype A1) in healthy carriers also raised the concerns about the potential transmission to other bird species.

3.2. New species: *Trichomonas gypaetinii*

3.2.1. *Introduction*

As a consequence of an epidemiological study of oropharyngeal trichomonads carried out on wild birds admitted to wildlife recovery centers from Spain, a new species of trichomonad was described from cinereous (*Aegypius monachus*) and Egyptian vultures (*Neophron percnopterus*, Martínez-Díaz *et al.*, 2015). Its name was assigned as *T. gypaetinii*, due to its presence in birds from the subfamily Gypaetinae (Old World vultures).

3.2.2. *Host spectrum*

T. gypaetinii has been reported from cinereous vultures, Egyptian vultures, bearded vultures (*Gypaetus barbatus;* Grabensteiner *et al.*, 2010) and one American bald eagle (*Haliaeetus leucocephalus*; Kelly-Clark *et al.*, 2013).

3.2.3. *Morphology*

Like *T. gallinae*, with an ovoid to pyriform cell body shape, smaller size (mean measures 8x6 µm for *T. gypaetinii vs*. 11x7 µm for *T. gallinae*), and a longer and prominent axostyle projection (Martínez-Díaz *et al.*, 2015; Mehlhorn *et al.*, 2009) (figure 4).

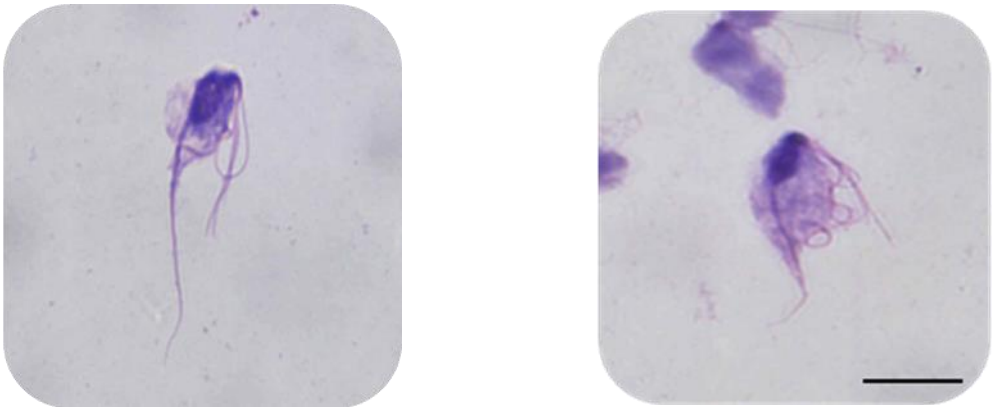

Figure 4. *T. gypaetinii* and *T. gallinae* trophozoites. Left: *T. gypaetinii*. Right: *T. gallinae*. Scale bars of 10 µm. Reproduced with permission from Martínez-Díaz *et al.*, 2015.

3.2.4. *Phylogenetic analysis*

T. gypaetinii shared 90-91% nucleotide homology with the ITS1/5.8S/ITS2 regions of *T. gallinae* isolates. This new organism was more similar to *T. stableri* and *T. vaginalis* strains (up to 97% for the previously cited genetic region) than to *T. gallinae* (Martínez-Díaz *et al.*, 2015).

3.3. New variants

Since 2001, new variants of oropharyngeal trichomonads from wild birds with more genetic homology to other trichomonads than to *T. gallinae* had been characterized (Table 1). The unraveling of the etiologic complex of avian trichomonosis helps to understand the epidemiology of the disease. These latest investigations denote that the boundaries between new species or subspecies are difficult to establish, with multiple genetic and morphological variables that need to be considered. In any case, further research will determine the extent and host distribution of these new variants or organisms.

Table 1. New species and variants of oropharyngeal trichomonads detected in birds.

Trichomonad new species /variants	**Host**	**Country**	**Reference**
T. vaginalis-like	Common ground-doves (*Columba passerina*)	USA	Gerhold *et al*., 2008
Trichomonas sp.	Mockingbirds (*Mimus polyglottos*)	USA	Anderson *et al.*, 2009
T. vaginalis-like (later *T. gypaetinii*)	Bearded vultures	Czech Republic	Grabensteiner *et al.*, 2010
T. tenax-like	Racing pigeons	Austria	Grabensteiner *et al.*, 2010
T. vaginalis-like	Striped owls (*Asio clamator*)	Brazil	Ecco *et al.*, 2012
Simplicomonas sp.	Green-winged saltators (*Saltator similis*)	Brazil	Ecco *et al.*, 2012
T. vaginalis-like (later *T. gypaetinii)*	Bald eagles (*Haliaeetus leucocephalus*)	Canada	Kelly-Clark *et al*., 2013
T. tenax-like	European turtle doves, woodpigeons and stock doves (*Columba oenas*)	UK	Lennon *et al*., 2013
T. stableri	Pacific Coast band-tailed pigeons	USA	Girard *et al.*, 2014a
T. blagburni-like	Pacific Coast band-tailed pigeons	USA	Girard *et al.*, 2014b
T. vaginalis-like (later *T. gypaetinii)*	Egyptian vultures	Spain	Martínez-Herrero *et al.*, 2014
T. canistomae-like	European turtle doves (*Streptopelia turtur*) and goshawks (*Accipiter gentilis*)	Spain	Martínez-Herrero *et al.*, 2014
T. gypaetinii	Cinereous and Egyptian vultures	Spain	Martínez-Díaz *et al.*, 2015
Trichomonas sp.	European turtle doves	UK	Stockdale *et al*., 2015

4. PRESENCE IN DIFFERENT GROUPS OF BIRDS

4.1. Order Accipitriformes

Diurnal raptors constitute one of the most affected groups of birds, due to their contact with columbiformes, the reservoir hosts of the protozoan, either as a food resource or at water stations (Purple *et al.*, 2015). Avian trichomonosis would probably be a major infectious disease for this group of birds since, probably, prehistoric times (Wolff *et al.*, 2009).

Ornithophagous birds of prey, especially those that include columbiformes on their diets, are at a higher risk of exposure and infection. For instance, Wieliczko *et al.* (2003) found a 100% infection rate in goshawk nestlings of Poland, and Krone *et al.* (2005) found a 65.1% prevalence in Berlin's nests with a low rate of lesions. Also, 85% of Cooper's hawk (*Accipiter cooperii*) nestlings were infected in Arizona (Urban *et al.*, 2014). Later, Martínez-Herrero *et al.* (2014) reported that ornithophagous raptors were at a lower risk of suffering from avian trichomonosis in comparison with non-strict ornithophagous ones, probably due to a host-parasite adaptation.

Studies on wild, free-living raptors are still scarce and surveillance efforts are restricted mostly to threatened species. For example, nestlings of Bonelli's eagle (*Aquila fasciata*), an endangered species of the western Mediterranean basin, had 36% prevalence in one study of Spain (Real *et al.*, 2000) and 42.9% in Southern Portugal (Höfle *et al.*, 2000). Two publications highlighted that avian trichomonosis was the primary cause of admission for birds of prey due to infectious diseases in wildlife recovery centers in retrospective studies (Wendell *et al.*, 2002; Molina-López *et al.*, 2011) while Martínez-Herrero *et al.* (2014) found that 11.4% of predator birds admitted in several wildlife hospitals were infected.

Koyama *et al.* (1971) published the findings of *T. gallinae* in secretary birds (*Sagittarius serpentarius*), the only report on the species up to the current authors' knowledge.

4.2. Order Anseriformes

Descriptions of affected birds of this order are minimal. There is one report from farmed ducks that suffered from respiratory and intestinal involvement, with inflammation of the infraorbital sinuses, nasal cavity and trachea, with mucofibrino-purulent discharge that resembled the disease (Tsai *et al.*, 1997). However, *Tetratrichomonas anatis* was determined as the etiological agent with no further genetic characterization.

4.3. Order Columbiformes

Different levels of prevalence are found in the oldest reservoir host of *T. gallinae*: birds from the Order Columbiformes. For instance, McKeon *et al.* (1997) described 46% of infection in Senegal doves

(*Spilopelia senegalensis*) and Lennon *et al.* (2013) obtained 86% prevalence in European turtle doves (*Streptopelia turtur*) and Eurasian collared doves (*Streptopelia decaocto*). In contrast, 5.6% of infected mourning doves (*Zenaida macroura*) were reported by Schulz *et al*. (2005). Obviously, local and seasonal variations occur, but infection in columbiformes is described as endemic. Indeed, this group of birds is thought to be responsible of the worldwide distribution of the protozoan (revised in Forrester and Foster, 2008).

Epidemics with high mortality and morbidity that occur at periodic time intervals have been described in some species. This is the case of Pacific Coast band-tailed pigeons in USA (Rogers *et al.*, 2016a). The species was monitored in 1951 (Stabler) and lately, mortality events have been increasing. The negative effects of avian trichomonosis on the population dynamics have raised the concerns about the transmission of these new species or variants of oropharyngeal trichomonads among wild birds. Seasonality is also another factor that is frequently present in avian trichomonosis episodes of wild columbiformes. Some examples are: the massive mortality reported by Höfle *et al.* (2004) in a wintering population of woodpigeons (*Columba palumbus*) in Spain, the highest prevalence during winter and summer in Spanish rock pigeons (*Columba livia*) by Sansano-Maestre *et al.* (2009) or with mild temperatures and low rainfall in the case of Mauritian columbiformes (Bunbury *et al.*, 2007). In this last case, the endemic species was threatened by this infectious disease that increased their risk of extinction.

4.4. Order Falconiformes

This group of birds is commonly affected of trichomonosis due to their predatory habits. The ancient falconry technique implied that the contact with the protozoan might occur with high frequency. Indeed, Samour and Naldo (2003) diagnosed 393 of 7,085 cases, being 5.5% of the patients from the Falcon Specialist Hospital of Saudi Arabia.

In Spain, there are two studies from admissions to wildlife recovery centers. Sansano-Maestre *et al.* (2009) determined 12.5% prevalence in peregrine falcons (*Falco peregrinus*) and 10.3% in common kestrels (*Falco tinnunculus*). Later, Martínez-Herrero *et al.* (2014) reported 4.2% and 14.2% infection rates in the same hosts.

Besides, captive raptor breeding centers would be at risk of infection if a proper preventive management is not implemented (Forbes, 2016). Several measures, like control of food and water sources and avoidance of contact with wild birds, in addition to an individual proper diagnosis, are required (Kubiak and Forbes, 2016). In fact, Martínez-Herrero *et al.* (2014) found 4.4% prevalence in a lesser kestrel (*Falco naumanni*) captive breeding center, with animals sub-clinically infected, without lesions and normal feeding behavior.

4.5. Order Galliformes

The name of *T. gallinae* derives from this group of birds, as the first description of this parasitic infection was made on them (Rivolta, 1878) although it is not commonly found in this type of birds. Poultry and turkeys could also suffer the disease, with studies reported from industrial production (Davidson *et al.*, 1985; Willoughby *et al.*, 1995; Mirzaei *et al.*, 2016). It seems a rare affection of wild gallinaceous birds.

4.6. Order Gruiformes

Bailey *et al.* (1996) reported avian trichomonosis in captive rufous-crested bustards (*Eupodotis ruficrista*). Columbiformes were the likely source of the infection, due to the uncontrolled access to the food and water farm supplies. The same etiology was determined in houbara bustards (*Chlamydotis undulata*) and Kori bustards (*Ardeotis kori*) with caseous inflammatory lesions at the oropharynx (Silvanose *et al.*, 1998).

4.7. Order Passeriformes

A notable importance and attention has been driven during recent years to avian trichomonosis in this group of birds. The disease was normally rare in wild passeriformes, although in 2002 an outbreak in house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*) was reported in the USA (revised in Forrester and Foster, 2008). In 2009, Anderson *et al.*, informed about positive cases in house finches, several corvids species (scrub jays-*Aphelocoma californica*, crows-*Corvus brachyrhynchos* and ravens-*Corvus corax*) and mockingbirds attended in a wildlife recovery center of California. Moreover, in 2010 Anderson *et al.* published that avian trichomonosis reached the level of emerging disease for house finches. Other reports were informing about the disease in Canadian finches: American goldfinches (*Carduelis tristis*) and purple finches (*Carpodacus purpureus*; Forzán *et al.*, 2010).

In the UK this group of birds suffered a massive epizootic of the disease (Pennycott *et al.*, 2005; Lawson *et al.*, 2006; Duff *et al.*, 2007; Robinson *et al.*, 2010). Carcasses were recovered from gardens and submitted for examination. A 35% and 21% decrease was observed in the population of greenfinches (*Chloris chloris*) and chaffinches (*Fringilla coelebs*), respectively, during next years. The first cases occurred in 2005-06 and extended to European countries in subsequent years: Sweden, Norway, Finland, France, Austria and Slovenia (Neimanis *et al.*, 2010; Gourlay *et al.*, 2011; Ganas *et al.*, 2014). Avian trichomonosis was determined as a novel emergent disease for wild birds, with unknown consequences in the population dynamics of raptor and other sympatric bird species (Robinson *et al.*, 2010; Chi *et al.*, 2013; Zu Ermgassen *et al.*, 2016).

The likely route of infection for passeriformes was determined to be, mainly, through the ingestion of contaminated food or water in areas shared with columbiformes (Robinson *et al.*, 2010).

4.8. Order Psittaciformes

Mostly psittaciformes kept as pets are reported with cases of avian trichomonosis. Lumeij (1994) informed about cases in Amazon parakeets (*Amazona* spp.) and conure species such as Aratinga parakeets (*Aratinga* spp.). The popular budgerigars (*Melopsittacus undulatus*) were the subject of an investigation at their native country, Australia. Researchers stated 11.4% prevalence from captive flocks (McKeon *et al.*, 1997). Later, an exhaustive revision of clinical cases was done by Park (2011). This author described the disease from 146 psittaciformes, including budgerigars, rainbow lorikeets (*Trichoglossus haematodus*), purple-crowned lorikeets (*Glossopsitta porphyrocephala)* and cockatiels (*Nymphicus hollandicus*).

4.9. Order Strigiformes

Nocturnal birds of prey are another frequently affected group of birds. Obviously, the diet of the species will determine the likelihood of the infection. Several studies have described the lesions and pathology of fatal cases (Tanabe, 1925; Jessup, 1980; Schulz, 1986; Pokras *et al.*, 1993; Sansano-Maestre *et al.*, 2009; Ecco *et al.*, 2012; Martínez-Herrero *et al.*, 2014; Rogers *et al.*, 2016b).

In USA, Schulz (1986) investigated the occurrence of the parasite among barn owls (*Tyto alba*) and stated that trichomonosis was the most prevalent infectious disorder. In Spain, 14% prevalence was reported from a wildlife recovery center (Sansano-Maestre *et al.*, 2009). The parasite has been recovered from even Eurasian scops-owls (*Otus scops*), a 16-20 cm body-length species of 60-135 weight grams, with a mainly insectivorous diet (Martínez-Herrero *et al.*, 2014).

4.10. Other orders with sporadic reports

In 1938, Hees described a case in a gull (Order Charadriiformes) in northern UK (revised in Forrester and Foster, 2008).

5. CLINICAL SIGNS AND LESIONS

Clinical presentation of avian trichomonosis usually consists in a nonspecific symptomatology. Birds appear in a low body condition, with ruffled feathers and lethargic behavior, anorexia, reluctance to fly and depression (figure 5). Feathers around oral cavity and nostrils may appear stuck to the beak as result of regurgitation. Diarrhea, when present, is often characterized by yellow pasty stools. Some animals may display respiratory signs such as labored breathing, sinusitis and nasal discharge (Krone *et al*., 2005; Anderson *et al*., 2010; Neimanis *et al*., 2010; Madani *et al*., 2015; Forzán *et al*., 2016).

Lesions of avian trichomonosis are usually restricted to upper digestive system, including oropharyngeal cavity, esophagus and crop. The absence of cystic forms of the trophozoite prevents the

possibility of resistance to the acidic medium of the proventriculus and thus, the digestive tract below is rarely affected. However, infection of upper and low respiratory airways is possible, and could represent a diagnostic challenge for the veterinarian (Samour and Naldo, 2001).

The formation of yellow-white nodules and plaques in the oral cavity are characteristics of avian trichomonosis but are not pathognomonic lesions. Vitamin A deficiency, candidiosis, poxvirus infection, aspergillosis, capillariosis and bacterial stomatitis might display similar gross appearance.

Lesions grow in a few days since the infection is established. At first, trophozoites attach to the epithelial cells inducing a damage, which leads to a separation and removal of the cells. The disruption of the epithelial layer favors the entrance of trichomonads and opportunistic bacteria to deeper epithelial layers. The cellular reaction of the immune system of the bird aids in the formation of an early stage granuloma.

Initial oral lesions are white, ulcerative and are located at the oropharynx (Krone *et al.,* 2005). As the disease progresses, multiple new ulcerated areas may appear and coalesce. The infiltration of inflammatory cells (plasma cells and macrophages) surrounding the affected area causes the formation of caseo-necrotic granulomas. Chronic granulomas usually are larger than the tracheal opening, show a characteristic yellow coloration and may spread to the esophagus and crop (figure 6) (Krone *et al.,* 2005; Anderson *et al.,* 2010; Borji *et al.,* 2011; Ecco *et al*., 2012; Rogers *et al.,* 2016). In rare cases, granulomas may extend to the choanae, sinuses, and inner ear as well as the bone at the base of the skull (Ecco *et al*., 2012; Kunca *et al.,* 2015; Rogers *et al.,* 2016). Some of these granulomas can eventually block the oral opening and prevent the intake of food and water or even cause the death by asphyxia or suffocation (Real *et al.,* 2000; Samour and Naldo, 2001). These gross findings appear frequently in raptors and pigeons with chronic trichomonosis. Passerines rarely present these macroscopic lesions in the lumen of the oropharynx, which difficult the presumptive diagnosis. Domestic pigeons with macroscopic oral granulomas frequently sustain lesions in other organs such as trachea, lungs, liver and kidney (Borji *et al.,* 2011; El-Khatam *et al.,* 2016).

Histologically there is a generalized congestion and edema of affected tissues. Yellow granulomas correspond with caseous necrosis areas and surrounding tissues may develop coagulative necrosis. Passeriformes frequently develop an epithelial hyperplasia of the esophagus and crop with the consequent thickening of the mucosa (Anderson *et al.,* 2010). The epithelium has multifocal areas of lymphoplasmacytic, heterophilic, granulomatous or necrotizing esophagitis associated with bacteria (Ecco *et al*., 2012; Girard *et al,* 2014a). Similar lesions are observed in the epithelium of the trachea (Borji *et al.,* 2011; Madani *et al.,* 2015; El-Khatam *et al.,* 2016). When present, lesions in the lungs are characterized by congestion and inflammatory cell infiltration in the bronchial wall and in the peri-bronchial tissue. The liver shows focal areas of necrosis with bile duct epithelial hyperplasia and vacuolar degeneration of hepatocytes. Renal tubular necrosis and multiple foci of mononuclear inflammatory cells are the most consistent pathologic findings in renal tissues.

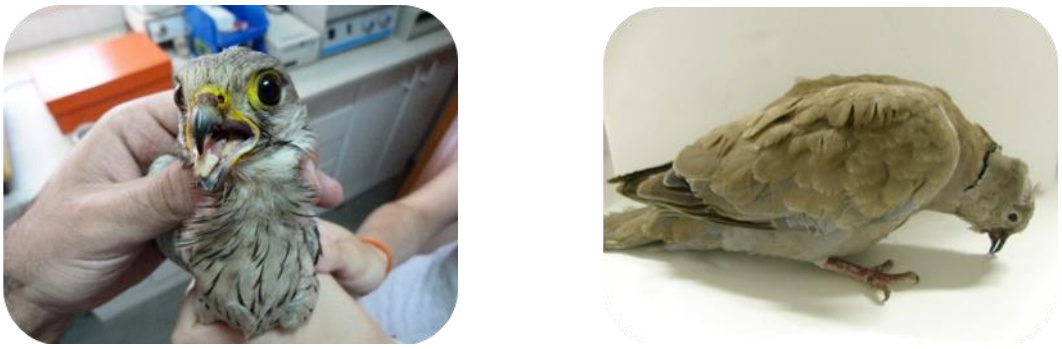

Figure 5. Clinical signs of avian oral trichomonosis. Left: stuck feathers around nostrils and oral cavity in a common kestrel (*Falco tinnunculus*). Right: reluctance to fly and apathy in a Eurasian collared dove (*Streptopelia decaocto*).

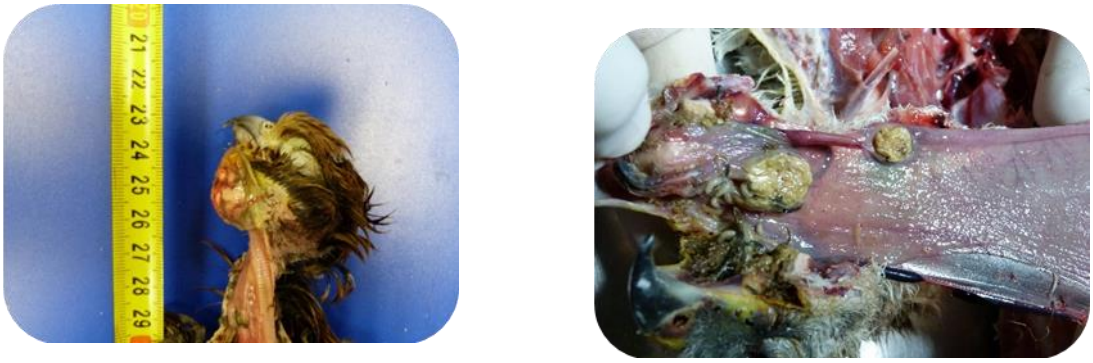

Figure 6. Yellow-white nodules and plaques in the oral cavity of a common kestrel (*Falco tinnunculus*) and a Bonelli's eagle (*Aquila fasciata*).

6. DIAGNOSIS

Although clinical signs and pathological findings are very suggestive of avian trichomonosis, the confirmation and identification of the species should be performed in the laboratory.

The classical method to diagnose avian oropharyngeal trichomonosis consists in wet-mount cytology. This is a non-expensive test in which the sample, taken from the oropharyngeal cavity and crop with a swab moistened in physiological saline solution, is extended on a microscopic slide. The characteristic movement of trophozoites facilitates their detection. The inconvenience of this method is a low sensitivity (more than two times lower) when compared with the culture (Bunbury *et al*., 2005) and the examination of the slide should be performed immediately after obtaining the sample, which makes difficult its application in the field. Cytologies stained with a metachromatic method, such as Diff-Quick

or Giemsa, has been used. However, the risk of false negative diagnosis is high, especially early at the infection when low trophozoite numbers may be present.

Cultures of oropharyngeal trichomonads in specific media is regarded as the most sensitive method for the diagnosis of the infection (Wieliczko *et al*., 2003; Krone *et al*., 2005; Gerhold *et al*., 2008; Sansano-Maestre *et al*., 2009; Grabensteiner *et al*., 2010; Zimre-Grabensteiner *et al*., 2011; Chi *et al*., 2013; Girard *et al*., 2014a and b; Martínez-Herrero *et al*., 2014; Kunca *et al*., 2015; Martínez-Díaz *et al*., 2015; McBurney *et al*., 2015). The main advantage of parasite culture is that it can be used as a transport medium from the field to the laboratory, facilitating the detection of the trophozoites. A commercial kit for the diagnosis of *Tritrichomonas foetus* from cattle (InPouch® TF, Biomed Diagnostics, San Jose California EEUU) is available and can be used for the diagnosis of avian trichomonosis.

Polymerase chain reactions (PCR) of different loci (ITS1/5.8S/ITS2 region, α-tubulin gene, small rRNA subunit, Fe-hydrogenase gene) have been used in order to confirm the presence of parasite DNA (in carcass samples and cultures). PCR analysis is mandatory for the identification of the species taking into consideration the complex etiology of oropharyngeal bird trichomonosis (Höfle *et al*., 2004; Villanúa *et al*., 2006; Gaspar da Silva *et al*., 2007; Gerhold *et al*., 2008; Grabensteiner *et al*., 2010; Lawson *et al*., 2011; Chi *et al*., 2013; Ganas *et al*., 2014; Girard *et al*., 2014a; Martínez-Herrero *et al*., 2014; Martínez-Díaz *et al*., 2015). Recently, FTA cards were used to preserve DNA from DNases and other degrading processes. In that way, subsequent molecular analysis would be easier to perform from samples taken in the field, which would be a great advantage (Holt *et al*., 2015). Unfortunately, there are no studies comparing PCR results from oropharyngeal swabs with cultures and its validation as a diagnostic method.

7. TREATMENT AND PREVENTION

Different drugs have been proved effective for the treatment of avian trichomonosis. For decades, nitroimidazole drugs were the treatment of choice in affected birds. Metronidazole, dimetridazole, ipronidazole, ronidazole and carnidazole have been successfully used in different species of birds, directly or mixed with food or drinking water (Lumeij and Zwijnenberg, 1990). Among them, dimetridazole is a drug with a lower therapeutic index in birds, inducing hepatoxicity in passerines, psittacines and fledglings. The indiscriminate use of sub-therapeutic doses in racing pigeons has leaded to the development of resistant strains to these drugs. Special care should be taken when administering medications in food and drinking water, as sick birds have reduced or altered intake, with a consequent sub-medication and treatment failure, which can trigger the development of resistance (Lumeij and Zwijnenberg, 1990; Muñoz *et al.*, 1998; Zimre-Grabensteiner *et al*., 2011).

Since sick animals have poor body condition, it is mandatory to correct the electrolytic balance and the catabolic state with supplementary energy sources. Wide-spectrum antibiotic drugs should be used in order to prevent secondary bacterial infections. Oral and nasal flushing with topical disinfectants, such as 0.5% chlorexidin, could reduce the microbial charge. In some cases surgical scission of the granuloma may be necessary.

Recently, extracts of *Artemisia sieberi* and garlic plants (*Allium* spp.) have proved their efficacy against *T. gallinae*, which promises a new source of anti-trichomonad agents that could be used as preventive and therapeutic drugs (Seddiek *et al.*, 2014; Yussefi *et al.*, 2017).

In the absence of vaccines and drugs as effective preventive treatments, the infection can be controlled by avoidance of elevated concentrations of birds (especially columbiformes) at food stations and drinkers, implementing disinfection protocols and replacing food frequently at these points.

8. FACTORS THAT TRIGGER PARASITE PATHOGENICITY

As we have mentioned before, infections by *T. gallinae* are frequent in some avian species or in certain populations living under circumstances that favor the spread of the disease. In columbiformes, most of the infected animals show no apparent clinical signs of disease, or might suffer subclinical infections (<1% in Sansano-Maestre *et al.*, 2009; 1.9% in Bunbury *et al.*, 2007 as examples). Only a small percentage of them develop macroscopic lesions or die. This situation has led to some skeptical parasitologists to doubt about the pathogenic character of this protozoon. Still, we have to think that a similar situation occurs in many of the pathogens infecting animals. We do not doubt on the pathogenic character of *Salmonella* and, if we isolate it from an apparently healthy animal, we call it "healthy carrier". A similar situation occurs with trichomonads in general. As far as we know, some species of trichomonads can be infecting the host during several months: *T. vaginalis* and *T. foetus* (revised in Hirt *et al.*, 2002; Tolbert and Gookin, 2016). The same situation has been described in *T. gallinae* infected birds, in which the parasite can persist in a host for at least 20 months (Bunbury *et al.*, 2007).

Although the idea of "healthy carrier" for *T. gallinae* is being increasingly accepted nowadays among protozoologists, we are giving some hints trying to explain the situation in which the presence of *T. gallinae* is not accompanied by the appearance of lesions. Risk factors for the development of the disease associated with the host (host susceptibility, immune response, stress situations) or associated with the parasite (genotype associated virulence) are analyzed.

Little has been studied on the sub-clinical effects of the infection and some authors support the idea that a reduced survival rate in columbiformes is a consequence of *T. gallinae* infection (Bunbury *et al.*, 2007). A different situation has been described in other avian groups, mainly raptors, but specially during the last decade also fringilids, in which the infection is highly associated with the presence of

macroscopic lesions and even with mortality events in bird populations (Sansano-Maestre *et al.*, 2009; Lawson *et al.*, 2011; revised in Amin *et al.*, 2012; Martínez-Herrero *et al.*, 2014).

Since many of the surveys investigating the presence of *T. gallinae* in birds are cross-sectional studies, it is difficult to have a picture on the progression of the infection. It is possible that the infection occurred recently and there are no lesions present, but it could also be possible that the infection (and disease) was old and the parasite still persists even when evident signs of the disease are not present any longer. Bunbury *et al.* (2007) screened a population of the endangered Mauritian pink pigeon (*Nesoenas mayeri*) for two years and found a high prevalence rate with a low percentage of animals with active signs of trichomonosis, but suggested that a prolonged infection with the parasite, even without evident clinical signs, might affect the oncoming survival rate of the birds. Prevalence was higher with age, a fact that supports the hypothesis of long carrier status of previously infected animals. Lennon *et al.* (2014) observed that although the percentage of animals hosting *T. gallinae* was higher in adult columbiformes, the percentage of animals with lesions was higher in young individuals.

The high rate of infection accompanied by a low percentage of animals with clinical signs, and the relationship between prevalence rate and host age is a regular observation under a stable endemic situation. However, when an outbreak occurs, there is a high percentage of birds with clinical signs among the infected individuals in a short period of time. This situation can appear in naïve animals or in previously exposed animals under circumstances that favor the spread of the disease by reduction of the immune responses. Migration, food stress, climatic conditions, breeding season, sharing of water or food sources with columbiformes, are some examples (Keppie and Braun, 2000; Höfle *et al.*, 2004; Robinson *et al.*, 2010; Lawson *et al.*, 2011; Lennon *et al.*, 2014; McBurney *et al.*, 2015; Stockdale *et al.*, 2015).

The parasite can be pathogenic under certain circumstances, and risk factors are needed for the development of the disease. If those factors are not present, the disease will not develop. In this sense, a cross sectional study was published by Martínez-Herrero *et al.* in 2014 pointing out some of the factors than can influence the development of lesions in infected animals. Among them, the diet type in raptors, and the parasite genotype were more relevant than other factors, as Sansano-Maestre *et al.* (2009) previously suggested. When raptors feed exclusively on birds, lesions due to trichomonosis were less frequent. This situation can be explained by an efficient response of the immune system, and if the animal has frequent contact with the parasite, it gets refractory to the development of disease, even if the animal maintained the infection for a long time. In this sense, several authors have observed an inverse relationship between prevalence rate and percentage of animals showing gross lesions (Bunbury *et al.*, 2007; Martínez-Herrero *et al.*, 2014; Kunca *et al.*, 2015).

Risk factors associated with the parasite, such as genotype, have been analyzed, since many genetic variants have been described. Some of them can be pathogenic while others are not, as it has been described for other protozoa (*Eimeria* spp., *Cryptosporidium* spp. as examples). Many of the genetic variants have never been associated with disease. Others, however, have been found when

lesions are present and they might appear as single or mixed infections (Sansano-Maestre *et al.*, 2009; Grabensteiner *et al.*, 2010; Girard *et al.*, 2014b; Martínez-Herrero *et al.*, 2014). Since there are many variants without enough epidemiological and clinical data, we have focused in the two most frequent genetic groups of *T. gallinae*: ITS-OBT-Tg-1 (firstly described by Gerhold *et al.* in 2008 as genotype A), and ITS-OBT-Tg-2 (firstly described by Gerhold *et al.* in 2008 as genotype C). Both have been detected in animals with lesions, which means that both have the potential to induce disease. This pathogenicity has been demonstrated by *in vitro* virulence assays in cell cultures by Amin *et al*. (2012). In the study, the authors showed how different genotypes of *T. gallinae* induce cytopathogenic effect on the cells while *Tetratrichomonas gallinarum* did not provoke the same effect. More *in vitro* studies are required to clarify this issue. Perhaps the point is to focus on the virulence degree of the different genotypes instead of the strict categorization of pathogenic/non-pathogenic.

An association between genotype ITS-OBT-Tg-1 and the presence of gross lesions (including fatal cases) have been observed for the first time by Sansano-Maestre *et al*. in 2009, and later on by other authors including more avian species (Chi *et al.*, 2013). In 2015, McBurney *et al*. found that finches and rock pigeons with clinical signs of trichomonosis carried the same genotype (ITS-OBT-Tg-1), while healthy animals carried the other genotype (ITS-OBT-Tg-2). Stockdale *et al.* in 2015 detected four genetic variants of *T. gallinae* in European turtle doves, but all birds with clinical signs carried genotype ITS-OBT-Tg-1. Although this genotype has also been detected in animals without clinical signs, it might be possible that they had suffered the disease in the past (Zu Ermgassen *et al.*, 2016).

Also, the number of fatal cases or cases with gross lesions in which only genotype ITS-OBT-Tg-2 have been detected is much lower. The genotype of *T. gallinae* is associated with the bird species, although the most frequent genotypes have been detected in a wide variety of them. In Europe, genotype ITS-OBT-Tg-2 is highly associated with columbiformes and in some studies up to 100% of the animals carry the parasite (Lennon *et al*., 2013, 100% incidence in adult European turtle doves). Mixed infections (including more than one genotype) have been reported by different authors and it could be possible that when a mixed infection is present only one genotype is detected by culture or PCR-sequencing. To solve that problem, a direct amplification and sequencing of the swabs obtained from lesions is recommended. The only study involving genotype ITS-OBT-Tg-2 with the development of lesions was done in columbiformes in Egypt (El-Khatam *et al.*, 2016), in which genotype ITS-OBT-Tg-2 seems to appear more frequently, especially in pigeon farms. In this study, only one genotype of the parasite was found, and it seemed that a small percentage of animals developed lesions. Whereas genotype ITS-OBT-Tg-2 could also produce some degree of lesions, a control group of animals infected with genotype ITS-OBT-Tg-1 is lacking and for that reason it is difficult to obtain a clear conclusion from the study.

A question that has still not been investigated in depth is the association of genotype with a categorization of the disease; that is, cases of lethality, internal organs affected, severity of oropharyngeal lesions, and so on.

9. RECENT ADVANCES AND FUTURE STUDIES

We have seen during the past years how the diversity of avian oropharyngeal trichomonads has increased. The description of new species, probably circumscribed to certain hosts, is one of the issues that will be investigated and enrich our knowledge of avian trichomonosis in the near future.

Another fact that needs more clarification is which characteristics, if any, are associated with the genotype of the parasite. For example, is there a similar growth rate between both genotypes?. Do they need the same nutrients to grow *in vitro*?. This is an important issue, since the necessity of nutrients can explain differences in isolation success, or even in the virulence of the isolates. In this regard, the effect of certain nutrients on the growth of different genotypes of the parasite needs further investigation. The isolation and maintenance of certain genetic variants in the laboratory, under our experience, is not easy. If we rely solely on culture for epidemiological studies, then we may exclude some genetic variants which grow slower or do not grow at all in the most frequent employed culture media.

In order to optimize the results obtained in epidemiological studies, a consensus diagnostic technique among laboratories, sensitive enough to detect distinct species and low number of protozoa will be desirable. In this sense, it can be possible that PCR carried out on DNA isolated directly from oral swabs is a good option if targets with tandem sequence repeats are used (ITS1/5.8S/ITS2 region and small rRNA subunit). The possibility of sequencing and BLAST analysis of positive results is also an advantage if we want to obtain a detailed picture of the epidemiological situation.

The virulence of the isolates needs more *in vitro* and *in vivo* evidences. Although several studies have focused on the higher virulence of genotype ITS-OBT-Tg-1, a small percentage of animals infected with genotype ITS-OBT-Tg-2 and other less frequent species (such as *T. stableri*) develop lesions as well. An important fact that has to be present to validate epidemiological studies is that the wide spread genotypes are isolated from the same bird species. If only one of them is isolated (genotype-host association) it will be difficult to extract any conclusion. *In vitro* virulence assays using both genotypes are necessary to clarify the pathogenicity associated with the genotype of the isolate.

The virulence mechanisms of the parasite have not been elucidated yet. Although a couple of studies identified several proteins present in *T. gallinae*, which are similar to some virulence factors previously described in *T. vaginalis*, more studies focusing on the proteomic composition of the parasite are needed. The presence of cystein peptidases or GADPH are some of the above mentioned factors

(González-Díaz *et al.*, 2011; Amin *et al.*, 2012), but there are, still, many proteins to be described in this protozoan.

The mechanisms of transmission of the parasite can be direct, by feeding on infected preys or by feeding the young or during courtship, but they can also be indirect, when sharing of contaminated food or water is a common practice among birds. The role of pseudocysts in disease transmission has been poorly investigated (Tasca and De Carli, 2003), although several studies focused on the persistence of the parasite on food or water have been carried out (Kocan, 1969b; Kietzmann, 1990; Erwin *et al.*, 2000; Bunbury *et al*., 2007; Purple and Gerhold, 2015; Purple *et al.*, 2015). Description of survival and mechanisms of development of pseudocysts will be a valuable contribution to the epidemiology of the disease.

Another factor that has not been investigated in depth so far is the presence of concomitant flora that can exacerbate the disease. The presence of *Pseudomonas aeruginosa* has been pointed out as a factor that complicates the lesions (Samour, 2000). However, more studies including a general view of the flora present in healthy and infected populations of birds (metagenomics) are needed in order to clarify the influence of the microbiota on the progression of the disease. Also, the possibility of gene transferring among microorganisms present in the mucosae could be investigated. This idea has been suggested by some authors who found similar surface protein families in individuals colonizing mucosal surfaces (Hirt *et al*., 2002).

In order to control the disease, a mechanism of immunization of naïve birds under management or the development of a vaccine will be a great contribution, especially for endangered species in which oropharyngeal trichomonads constitutes a menace for them.

REFERENCES

Anderson, N.L., Grahn, R.A., Van-Hoosear, K., Bondurant, R.H. (2009). Studies of trichomonad protozoa in free ranging songbirds: prevalence of *Trichomonas gallinae* in house finches (*Carpodacus mexicanus*) and corvids and a novel trichomonad in mockingbirds (*Mimus polyglottos*). *Vet. Parasitol*., *161*, 178-186.

Anderson, N.L., Johnson, C.K., Fender, S., Heckly, S., Metzler, M., Nave, P. & Yim, J. (2010). Clinical signs and histopathologic findings associated with a newly recognized protozoal disease (*Trichomonas gallinae*) in free-ranging house finches (*Carpodacus mexicanus*). *J. Zoo. Wildl. Med*., *41*, 249-254.

Amin, A., Nöbauer, K., Patzl, M., Berger, E., Hess, M. & Bilic, I. (2012). Cysteine peptidases, secreted by *Trichomonas gallinae*, are involved in the cytopathogenic effects on a permanent chicken liver cell culture. *PLoS One*, *7*, e37417.

Bailey, T.A., Nicholls, P.K., Samour, J.H., Naldo, J., Wernery, U. & Howlett, J.C. (1996). Postmosterm findings in bustards in the United Arab Emirates. *Avian Dis., 40,* 296-305.

Boal, C.W., Manna, R.W. & Hudelson, K.S. (1998). Trichomoniasis in Cooper´s hawks from Arizona. *J. Wildl. Dis*., *34*, 590-593.

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*, 186-9.

Borror, D.J. (1988). *Dictionary of word roots and combining forms* (second edition). Mayfield Publishing Company, California, USA.

Brugerolle, G., Lee, J.J. (2000). Phylum Parabasalia. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.). *An Illustrated Guide to the Protozoa* (second edition, 1196-1250). Lawrence, Kansas, USA. ST: Society of Protozoologists, Blackwell Publishers.

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. (2007). *Trichomonas gallinae* in Mauritian columbids: implications for an endangered endemic. *J. Wild. Dis., 43,* 399-407.

Castro, C., Menna-Barreto, R.F., Fernandes, N.S., Saboia-Vahia, L., Dias-Lopes, G., Britto, C., Cuervo, P., de Jesus, J.B. (2016). Iron-modulated pseudocyst formation in *Tritrichomonas foetus*. *Parasitology*, *143*, 1034-1042.

Cepicka, I., Hampl, V. & Kulda, J. (2010). Critical taxonomic revision of Parabasalids with description of one new genus and three new species. *Protist., 161,* 400-433.

Chi, J.F., Lawson, B., Durrant, C., Beckmann, K., John, S. Alrefaei AF, Kirkbride K, Bell DJ, Cunningham, A.A. & Tyler, K.M. (2013). The finch epidemic strain of *Trichomonas gallinae* is predominant in British non-passerines. *Parasitol., 140,* 1234-1245.

Davidson, W.R., Nettles, V.F., Couvillion, C.E. & Howerth, E.W. (1985). Diseases diagnosed in wild turkeys (*Meleagris gallopavo*) of the southeastern United States. *J. Wildl. Dis*., *21*, 386-390.

Duff, J.P., Pennycott, T.W., Willmington, J.A. & Robertson, S.I. (2007). Emergence of garden bird trichomonosis. *Vet. Rec*., *161,* 828.

Ecco, R., Preis, I.S., Vilela, D.A., Luppi, M.M., Malta, M.C., Beckstead, R.B., Stimelmayr, R. & Gerlhold, 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.

Erwin, KG, Kloss, C., Lyles, J., Felderhoff, J., Fedynich, A.M., Henke, S.E. & Roberson, J.A. (2000). Survival of *Trichomonas gallinae* in white-winged dove carcasses. *J. Wildl. Dis*., *36*, 551-554.

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.

Forbes, N.A. Disease control and rehabilitation in a collection of raptors. 2016. Available from: URL: http://www.gwexotics.com/wp-content/uploads/2016/04/Disease-control-and-rehabilitation-raptors.pdf

Forrester, D.J. & Foster, G.W. (2008). Trichomonosis. In: Atkinson, C.T., Thomas, N.J., Hunter, D.B. (Eds.), *Parasitic diseases of wild birds* (pp 120-153). Wiley-blackwell, John Wiley and Sons Ltd. Ames, Iowa, USA.

Forzán, M.J., Vanderstichel, R., Melekhovets, Y.F. & McBurney, S. (2010). Trichomoniasis in finches from the Canadian Maritime provinces-An emerging disease. *Can Vet J*., *51*, 391-6.

Friedhoff, K.T., Kuhnigk, C. & Müller, I. (1991). Experimental infections in chickens with *Chilomastix gallinarum*, *Tetratrichomonas gallinarum* and *Tritrichomonas eberthi*. *Parasitol. Res*., *77*, 329-334.

Ganas, P., Jaskulska, B., Lawson, B., Zadravec, M., Hess, M. & Billic, I. (2014). Multi-locus sequence typing confirms the clonality of *Trichomonas gallinae* isolates circulating in European finches. *Parasitology, 141*, 652-661.

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.

Gerhold, R.W., Yabsley, M.J., Smith, A.J., Osteergard, E., Mannan, W., Cann, J.D. & Fischer, J.R. (2008). Molecular characterization of the *Trichomonas gallinae* morphologic complex in the United States. *J. Parasitol*., *94*, 1355-1341.

Girard, Y.A., Rogers, K.H., Gerhold, R., Land, K.M., Lenaghan, S.C. Woods, L.W., Haberkern, N., Hopper, M., Cann, J.D. & Johnson, C.K. (2014a). *Trichomonas stableri* n. sp., an agent of trichomonosis in Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*). *Int. J. Parasitol. Parasites Wildl*., *3*, 32-40.

Girard, Y.A., Rogers, K.H., Woods, L.W., Chouicha, N., Miller, W.A. & Johnson, C.K. (2014b). Dual-pathogen etiology of avian trichomonosis in a declining band-tailed pigeon population. *Infect. Genet. Evol*., *24*, 146-156.

Gourlay, P., Decors, A., Jouet, D., Treilles, D., Lemberger, K., Faure, E., Moinet, M., Chi, J., Tyler, K., Cunningham, A &Lawson, B. (2011). Finch trichomonosis spreads to France. *Europ. Sec. Wildl. Dis. Ass. Bull*., *2*, 9-10.

González-Díaz, H., Prado-Prado, F., García-Mera, X., Alonso, N., Abeijón, P., Caamaño, O., Yáñez, M., Munteanu, C.R., Pazos, A., Dea-Ayuela, M.A., Gómez-Muñoz, M.T., Garijo, M.M.,

Sansano, J., Ubeira, F.M. (2011). MIND-BEST: Web server for drugs and target discovery; design, synthesis, and assay of MAO-B inhibitors and theoretical-experimental study of G3PDH protein from *Trichomonas gallinae. J Proteome Res*., *10*, 1698-718.

Grabensteiner, E., Bilic, I., Kolbe & T., Hess, M. (2010). Molecular analysis of clonal trichomonad isolates indicate the existence of heterogenic species present in different birds and within the same host. *Vet. Parasitol*., *172*, 53-64.

Granger, L., Warwood, S.J., Benchimol, M. & de Souza, W. (2000). Transient invagination of flagella by *Tritrichomonas foetus*. *Parasitol. Res*., *86*, 699-709.

Hirt, R.P. Harriman, N., Kajava, A.V. & Embley, T.M. (2002). A novel potential surface protein in *Tritrichomonas vaginalis* contains a leucine-rich repeat shared by micro-organisms from all three domains of life. *Mol. Biochem. Parasitol*., *125*, 195-199.

Höfle, U., Blanco, J.M., Palma, L. & Melo, P. (2000). Trichomoniasis in Bonelli's Eagle (*Hieraaetus fasciatus*) nestlings in south-west Portugal. In J.T. Lumeij, J.D. Remple, P.T. Redig, M. Lierz, & J.E. Cooper (Eds.). *Raptor Biomedicine III* (pp. 45–51). Lake Worth, FL: Zoological Education Network.

Höfle, U., Gortazar, C., Ortíz, J.A., Knispel, B. & Kaleta, E.F. (2004). Outbreak of trichomoniasis in a woodpigeon (*Columba palumbus*) wintering roost. *Europ. J. Wildl. Res*., *50*, 73-77.

Holt, J.C., Purple, K.E. & Gerhold, R. (2015). Use of FTA technology for detection of *Trichomonas gallinae*. *Vet. Parasitol*., *212*, 396-399.

Honigberg, B.M., Becker, R.D., Livingston, M.C. & McLure, M.T. (1964). The behavior and pathogenicity of two strains of *Trichomonas gallinae* in cell cultures. *J. Protozool*., *11*, 447-465.

Honigberg, B.M., Brugerolle, G. (1990). Structure. In: Honigberg, B.M. (Ed.). *Trichomonads of veterinary importance in parasitic protozoa* (pp. 164-273). Academic Press, New York, USA.

Jaquette, D.S. (1950). Hepatic trichomoniasis in esophagotomized pigeons. *Poultry Science*, *29*, 157-158.

Jessup, D.A. (1980). Trichomoniasis in great horned owls. Mod. *Vet. Pract*., *61*, 601-604.

Kelly-Clark, W.K., McBurney, S., Forzán, M.J., Desmarchelier, M., Greenwood, S.J. (2013). Detection and characterization of a Trichomonas isolate from a rehabilitated bald eagle (*Haliaeetus leucocephalus). J. Zoo Wildl. Med*., *44*, 1123-1126.

Keppie, D.M. and Braun, C.E. 2000. Available from: URL: https://birdsna.org/Species-Account/bna/species/batpig1. DOI: 10.2173/bna.530. Accessed 23.03.2017.

Kietzmann, G.E. (1990). Transmission of *Trichomonas gallinae* to ring doves (*Streptopelia risoria*). *Proc. South Dakota Acad. Sci*., *69*, 95-98.

Kocan, R.M. (1969a). Different organ preferences by the same strain of *Trichomonas gallinae* in different host species. *J. Parasitol*., *55*, 1003.

Kocan, R.M. (1969b). Various grains and liquid as potential vehicles of transmission for *Trichomonas gallinae*. *Bull. Wildl. Dis. Ass*., *5*, 148-149

Kocan, R.M., Herman, C.M. (1971). Trichomoniasis. In: Davis, J.W., Anderson, R.C., Karstad, L., Trainer, D.O. (Eds.). *Infectious and parasitic diseases of wild birds* (pp. 282-290). Iowa State University Press, Ames, Iowa, USA.

Koyama, T., Kumada, M., Ochi, R., Nakagawa, S., Tashiro, K. (1971). *Trichomonas gallinae* found in secretary birds (*Serpentarius secretarius*). *Jap. J. Parasitol*., *20*, 495-497.

Krone, O., Altenkamp, R. & Kenntner, N. (2005). Prevalence of *Trichomonas gallinae* in northern goshawks from the Berlin area of northeastern Germany. *J Wildl Dis*., *41*, 304-9.

Kunca, T., Smejkalová, P. &. (2015). Trichomonosis in Eurasian sparrowhawks in the Czech Republic. *Folia Parasitol (Praha), 62*, 035.

Kubiak, M., Forbes, N.A. Common diseases in birds of prey-part 1. 2016. Available from: URL: http://www.gwexotics.com/wp-content/uploads/2016/04/Common-diseases-in-raptors-part-1.pdf

Lawson, B., Cunningham, A., Chantrey, J., Hughes, L., Kirkwood, J., Pennycot, T., Simpson, V. (2006). Epidemic finch mortality. *Vet. Rec*., *159*, 367.

Lawson, B.; Cunningham, A.A., Chantrey, J., Hugher, L.A., John, S.K., Bunbury, N., Bell, D.J., Tyler, K.M. (2011). A clonal strain of *Trichomonas gallinae* is the aetiologic agent of an emerging avian epidemic disease. *Infect. Genet. Evol*., *11*, 1638-1645.

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.

Lumeij, J.T. (1994). Gastroenterology. In: Ritchie, B.W., Harrison, G.J., Harrison, L.R. (Eds.). *Avian medicine: principles and application* (pp. 482-521). Wingers Publishing, Lake Worth, Florida, USA.

Lumeij, J.T. & Zwijnenberg, R.J. (1990). Failure of nitro-imidazole drugs to control trichomoniasis in the racing pigeon (*Columba livia domestica*). *Avian Pathol.*, *19*, 165–166.

Mariante, R.M., Lopes, L.C. & Benchimol, M. (2004). *Tritrichomonas foetus* pseudocysts adhere to vaginal epithelial cells in a contact-dependent manner. *Parasitol. Res*., *92*, 303-312.

Martínez-Díaz, R.A., Ponce-Gordo, F., Rodríguez-Arce, I., Martínez-Herrero, M.C., González-González, F, Molina-López, R.A. & Gómez-Muñoz, M.T. (2015). *Trichomonas gypaetinii* n. sp., a new trichomonad from the upper gastrointestinal tract of scavenging birds of prey. *Parasitol. Res*., *114*, 101-112.

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.

Mattern, C.F.T., Honigberg, B.M. & Daniel, A.W. (1973). Fine-structural changes associated with pseudocyst formation n *Trichomitus batrachorum*. *J. Protozool*., *20*, 222–229.

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.

McBurney S, Kelly-Clark WK, Forzán MJ, Lawson B, Tyler KM & Greenwood SJ. (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. *Parasitol., 142*, 1053-1062.

Mehlhorn, H., Al-Quraishy, S., Aziza, A. & Hess, M. (2009). Fine structure of the bird parasites *Trichomonas gallinae* and *Tetratrichomonas gallinarum* from cultures. *Parasitol. Res*., *105*, 751-756.

Mirzaei, M., Ghashqhaei, O. & Khedri, J. (2016). First report of an outbreak trichomoniasis in turkey in Sistan, Iran. *J. Parasit. Dis*., *40*, 61-64.

Molina-López, R.A., Casal, J. & Darwich, L. (2011). Causes of morbidity in wild raptors populations admitted at a wildlife rehabilitation Centre in Spain from 1995-2007: a long term retrospective study. *PLoS One, 6*, e24603.

Muñoz, E., Castella, J. & Gutierrez, J. F. (1998). *In vivo* and *in vitro* sensitivity of *Trichomonas gallinae* to some nitroimidazole drugs. *Vet. Parasitol., 78*, 239–246.

Nadler, S.A. & Honigberg, B.M. (1988). Genetic differentiation and biochemical polymorphism among trichomonads. *J. Parasitol., 74*, 797-804.

Narcisi, E.M., Sevoian, M. & Honigberg, B.M. (1991). Pathological changes in pigeons infected with a virulent *Trichomonas gallinae* strain (Eiberg). *Avian Dis*., *35*, 55-61.

Neimanis, A.S., Handeland, K., Isomursu, M., Agren, E., Mattsson, R., Hamnes, I.S., Bergsjø, B. & Hirvelä-Koski, V. (2010). First report of epizootic trichomoniasis in wild finches (family Fringillidae) in southern Fennoscandia. *Avian Dis*., *54*, 136-41.

Park, F.J. (2011). Avian trichomoniasis: a study of lesions and relative prevalence in a variety of captive and free-living bird species as seen in an Australian avian practice. *Aust. Vet. J*., *89*, 82-88.

Pennycott, T., Lawson, B., Cunningham, A., Simpson, V. & Chantrey, J. (2005). Necrotic ingluvitis in wild finches. *Vet. Rec*., *157*, 360.

Pereira-Neves, A., Ribeiro, K.C. & Benchimol, M. (2003). Pseudocysts in trichomonads – new insights. *Protist, 154*, 313-329.

Pérez-Mesa, C., Stabler, R.M. & Berthrong, M. (1961). Histopathological changes in the domestic pigeon infected with *Trichomonas gallinae* (Jones' barn strain). *Avian Dis*., *5*, 48-60.

Pokras, M.A., Wheeldon, E.B. & Sedgwick, C.J. (1993). Trichomoniasis in owls: report on a number of clinical cases and a survey of the literature. In: Redig, P.T., Cooper, J.E., Remple, J.D., Hunter,

D.B. (Eds.). *Raptor Biomedicine* (pp.88-91). University of Minnesota Press, Minneapolis, Minnesota, USA.

Purple, K.E. & Gerhold, R.W. (2015). Persistence of two isolates of *Trichomonas gallinae* in simulated bird baths with and without organic material. *Avian Dis*., *59*, 472-474.

Purple, K.E., Humm, J.M., Kirby, R.B., Saidak, C.G. & Gerhold, R. (2015). *Trichomonas gallinae* persistence in four water treatments. *J. Wildl. Dis*., *51*, 739-742.

Real, J., Mañosa, S. & Muñoz, E. (2000). Trichomoniasis in a Bonelli's eagle population in Spain. *J. Wildl. Dis*., *36*, 64-70.

Rivolta, S. 1878. Una forma di croup prodotta da un infusorio, nei polli. Giornale di Anatomia, Fisiologia e Patologia Animale, 10: 149-158. In: Forrester, D.J., Foster, G.W. (2008). *Trichomonosis. Parasitic diseases of wild birds* (pp.120). Atkinson, C.T., Thomas, N.J., Hunter, D.B. (Eds.). Wiley-blackwell, John Wiley and Sons Ltd. Ames, Iowa, USA.

Robinson, R.A., Lawson, B., Toms, M.P., Peck, K.M., Kirkwood, J.K. Chantrey J, Clatworthy IR, Evans AD, Hughes LA, Hutchinson OC, John SK, Pennycott TW, Perkins MW, Rowley PS, Simpson VR, Tyler KM, Cunningham AA. (2010). Emerging infectious disease leads to rapid population declines of common British birds. *PLoS One*, *5*, e12215.

Rogers, K.H., Girard, Y.A., Koenig, W.D. & Johnson, C.K. (2016a). Ecologic drivers and population impacts of avian trichomonosis mortality events in band-tailed pigeons(*Patagioenas fasciata*) in California, USA. *J. Wildl. Dis*., *52*, 484-494.

Rogers, K.H., Girard, Y.A., Woods, L. & Johnson, C.K. (2016b). Avian trichomonosis in spotted owls (*Strix occidentalis*): Indication of opportunistic spillover from prey. *Int J Parasitol Parasites Wildl.*, *5*, 305-311.

Samour, J.H. (2000). *Pseudomonas aeuriginosa* stomatitis as a sequel to trichomoniasis in captive saker falcons (*Falco cherrug*). *J. Avian Med. Surgery*, *14*, 113-117.

Samour, J. & Naldo, J. (2001). Trichomoniasis infections in captive falcons. *Exotic DVM*, *3*, 10-11.

Samour, J.H. & Naldo, J. (2003). Diagnosis and therapeutic management of trichomoniasis in falcons in Saudi Arabia. *J. Avian Med. Surg.*, *17*, 135-143.

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.

Schulz, T. (1986). Conservation and rehabilitation of the common barn owl. Proceedings of the National Wildlife Rehabilitators Association 4th Annual Meeting (NWRA). Brighton, Illinois, USA. pp: 146-166.

Schulz, J.H., Bermudez, A.J. & Millspaugh, J.J. (2005). Monitoring presence and annual variation of trichomoniasis in mourning doves. *Avian Dis*., *49*, 387-389.

Silvanose, C.D., Samour, J.H., Naldo, J.L. & Bailey, T.A. (1998). Oro-pharyngeal protozoa in captive bustards: clinical and pathological considerations. *Avian Pathol*., *27*, 526-530.

Seddiek, A., El-Shorbagy, M.M., Khater, H.F. & Ali, A.M. (2014). The antitrichomonal efficacy of garlic and metronidazole against *Trichomonas gallinae* infecting domestic pigeons. *Parasitol. Res., 113*, 1319-1329.

Sleigh, M.A. (1991). Flagellates and related protists. In: Sleigh, M.A. (Ed.). *Protozoa and other protists* (pp. 91-156). Cambridge University Press. London, Great Britain.

Stabler, R.M. (1938). *Trichomonas gallinae* (Rivolta, 1878) the correct name for the flagellate in the mouth, crop and liver of the pigeon. *J. Parasitol*., *24*, 553-554.

Stabler, R.M. (1941). The morphology of *Trichomonas gallinae* (=*columbae*). *J. Morph*., *69*, 501-509.

Stabler, R.M. & Engley, F.B. (1946). Studies on *Trichomonas gallinae* infections in pigeon squabs. *J. Parasitol*., *32*, 225-232.

Stabler, R.M. (1951). A survey of Colorado band-tailed Pigeons, mourning doves, and wild common pigeons for *Trichomonas gallinae*. *J. Parasitol*., *37*, 471-473.

Stabler, R.M. & Kihara J.T. (1954) Infection and death of the pigeon resulting from the oral implantation of single individuals of *Trichomonas gallinae*. *J. Parasitol*., *40*, 706.

Stachan, R., Nicol, C. & Kunstyr, I. (1984). Heterogeneity of *Tritrichomonas muris* pseudocysts. *Protistologica, 20*, 157- 163.

Stockdale, J.E., Dunn, J.C., Goodman, S.J., Morris, A.J., Sheehan, D.K., Grice, P.V., Hamer, K.C. (2015). The protozoan parasite *Trichomonas gallinae* causes adult and nestling mortality in a declining population of European Turtle Doves, *Streptopelia turtur*. *Parasitol., 142*, 490-498.

Tanabe, M. (1925). The cultivation of trichomonads from man, rat and owl. *J. Parasitol*., *12*, 101-104.

Tasca, T. & De Carli, G.A. (2003). Scanning electron microscopy study of *Trichomonas gallinae*. *Vet. Parasitol*., *118*, 37-42.

Tolbert, M.K. & Gookin, J.L. (2016). Mechanisms of *Tritrichomonas foetus* pathogenicity in cats with insights from venereal trichomonosis. *J. Vet. Intern. Med*., *30*, 516-526.

Tsai, S.S., Chang, T.C., Kuo, M. & Itakura, C. (1997). Respiratory and intestinal trichomoniasis in mule ducks. *Avian Pathol*., *26*, 651-656.

Urban, E.H. & Mannan, R.W. 2014. The potential role of oral pH in the persistence of *Trichomonas gallinae* in Cooper's hawks (*Accipiter cooperii*). *J. Wildl. Dis*., *50*, 50-55.

Vieira, P.B., Borges, F.P., Gottardi, B., Stuepp, C., Larré, A.B. Tasca, T., De Carli, G.A. (2008). Analysis of microtubule cytoskeleton distribution using a fluorescent taxoid in two trichomonadid protozoa: *Trichomonas gallinae* and *Tritrichomonas foetus*. *Exp. Parasitol*., *119*, 186-191.

Villanúa, D., Höfle, U., Pérez-Rodríguez, L. & Gortázar, C. (2006). *Trichomonas gallinae* in wintering common wood pigeons (*Columba palumbus*) in Spain. *Ibis*, *148*, 641-648.

Wendell, M.D., Sleeman, J.M., Kratz, G. (2000). Retrospective study of morbidity and mortality of raptors admitted to Colorado State University Veterinary Teaching Hospital during 1995 to 1998. *J. Wildl. Dis*., *38*, 101-106.

Willoughby, D.H., Bickford, A.A., Charlton, B.R., Cooper, G.L. (1995). Esophageal trichomoniasis in chickens. *Avian. Dis*., *39*, 919-924.

Wieliczko, A. Piaecki, T., Dorrestein, G.M., Adamski, A. & Mazrkiewicz, M. (2003). Evaluation of the health status of goshawk chicks (*Accipiter genitilis*) nestlings in Wroclaw vicinity. *Bull. Vet. Inst. Pullawy, 47*, 247-257.

Wolff, E.D.S., Salisbury, S.W., Horner, J.R. & Varricchio, D.J. (2009). Common avian infection plagued the Tyrant Dinosaurs. *PLoS One*, *4*, e7288.

Youssefi, M.R., Abouhosseini Tabari, M. & Moghadamnia, A.A. (2017). *In vitro* and *in vivo* activity of *Artemisia sieberi* against *Trichomonas gallinae. Iranian Journal of Veterinary Research, 18*, 25-29.

Zimre-Grabensteiner, E., Arshad, N., Amin, A. & Hess, M. (2011). Genetically different clonal isolates of *Trichomonas gallinae* obtained from the same bird can vary in their drug susceptibility, an *in vitro* evidence. *Parasitol. Int. 60*, 213-215.

Zu Ermgassen, E.K., Durrant, C., John, S., Gardiner, R., Alrefaei, A.F., Cunningham, A.A. & Lawson, B. (2016). Detection of the European epidemic strain of *Trichomonas gallinae* in finches, but not other non-columbiformes, in the absence of macroscopic disease. *Parasitology, 143*, 1294-1300.