**Title**: "Description and severity of lesions in avian oral trichomonosis with emphasis on wildlife recovery centers"

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**Abstract**

Avian trichomonosis is a parasitic disease caused by the flagellated protozoan *Trichomonas gallinae*. Columbiformes are the reservoir host of the parasite, with high levels of infection, but also other domestic and wild birds from a variety of orders are susceptible to the infection and development of gross lesions. A total of 94 clinical cases diagnosed of trichomonosis were selected for the categorization of their lesions at the upper digestive tract. The affected birds were classified into three different categories (mild, moderate and severe) based on the size, the depth and the location of the lesions. Mild grade is found in small and superficial lesions far from the oropharyngeal opening; moderate grade for bigger and deeper lesions, and severe grade for very big and deep lesions that impede swallowing or affect the skull. This revision of lesions will help to understand the pathologic and epidemiological information about avian trichomonosis. Furthermore, it will be helpful for the evaluation, prognosis and possible treatments among veterinarians and related professionals.

**Keywords**: *Trichomonas gallinae*, lesions, description, severity, avian trichomonosis

## 1. Introduction

Attending avian species is an expanding field of veterinary practice. In recent years, the number of birds has increased in both, veterinary clinics and rehabilitation centres. Previous studies indicate that the severity of injuries or diseases as well as poor body condition are the main prediction factors on mortality for birds (Molony et al., 2007, Molina-López et al., 2015). In this sense, it is essential to make a quick and accurate diagnosis, to establish a suitable prognosis and act accordingly.

Avian trichomonosis is one of the most common potentially fatal parasitic diseases in birds, caused by the flagellated protozoan *Trichomonas gallinae* (*Rivolta,* 1878). Pigeons and doves are the reservoir host of the parasite, with high levels of infection in domestic and wild birds, although birds from a variety of orders are susceptible to the infection and development of gross lesions (Mckeon et al., 1997; Samour and Naldo, 2003; Krone et al., 2005; Sansano-Maestre, 2009; Ecco et al., 2012; Stimmelmayr et al., 2012; Amin et al., 2014; Ganas et al., 2014; Martínez-Herrero et al., 2014; Madani et al., 2015; Zadravec et al., 2017; Feng et al., 2018). The direct life cycle of the flagellate favours the transmission between gregarious species of birds. Birds of prey are primarily affected after consumption of infected preys or scavenging on their carcasses. Recent epidemic outbreaks reported on wild finches had placed the status of emergent disease in several countries of Europe, Canada and North America (Amin et al., 2014; Quillfeldt et al., 2018).

The pathogenicity varies according to several factors, such as the number of previous infections, the health status and the immune response of the host and the particular strain of the parasite (Sansano et al., 2016; Martínez-Herrero et al., 2014, 2017).

Trophozoites initially start their multiplication by longitudinal binary fission at the surface of the mucosa and, as the infection progresses, deeper layers of the epithelium can be affected (Cole, 1999). The parasite frequently induces necrosis of the cells and granulomatous reaction of the involved tissues. Mild cases appear as small and superficial lesions, while in severe forms invasive granulomas invade the oropharyngeal cavity, esophagus and sinuses (Stabler, 1974; Cole, 1999). The extent and dimensions of these lesions impede the feeding or the breathing of the bird and subsequently lead to its death by starvation or suffocation if treatment is not administered, or secondary bacterial infections occur (Höfle et al., 2004; Chi et al., 2013; Lawson et al., 2011; Ganas et al., 2014).

Different patterns of distribution of macroscopical lesions have been described depending on the host order. Thus, in the order Columbiformes, the reservoir hosts of the parasite, caseonecrotic granulomas are the type of lesion that is mainly reported at the superior digestive tract: oropharyngeal cavity, crop and/or esophagus (Höfle et al., 2004; Hegemann et al., 2007; Sansano-Maestre et al., 2009; Stimmelmayr et al., 2012; Amin et al., 2014; Girard et al., 2014; Feng et al., 2018; Rogers et al., 2018). They appear as solid whitish-yellow lesions, focally or multifocally distributed that may coalesce and reach several centimeters of diameter in late stage infections. Internal organ involvement has been described, as lesions in the brain, conjunctive, myocardium, skeletal muscle, pancreas, kidneys, trachea, lungs, air sacs and especially in the liver, where they have been described in a similar way as caseonecrotic or decolorated areas (Pérez-Mesa et al., 1961; Höfle et al., 2004; Stimmelmayr et al., 2012; Girard et al., 2013, 2014; Stockdale et al., 2015). Bone and cartilage destruction has been referred by some authors in the skull and oropharyngeal cavity, in cases where lesions were located on the palate (Hegemann et al. 2007, Stimmelmayr et al., 2012).

In the order Accipitriformes, oropharyngeal caseonecrotic granulomas at the upper digestive tract are predominantly found, although invasion of the ocular and encephalic cavity has been also described (Real et al., 2000; Höfle et al., 2004; Krone et al., 2005; Martínez Herrero et al., 2019). Lesions in Falconiformes were found at the oropharynx, with caseous nodular proliferation, but also at the crop, esophagus, nasal cavity, infraorbital sinuses and the syrinx (Samour and Naldo, 2003). In Strigiformes, caseonecrotic granulomas tend to be located at the palate with involvement of the choanal slit and extension to the skull and cephalic sinuses, but also at the base of the pharynx (Pokras et al., 1993; Sansano-Maestre, 2009; Ecco et al., 2012; Niedringhaus et al., 2019). For Passeriformes, lesions tend to be circumscribed to the proximal esophagus, with necrotic foci of small (Forzán et al., 2010; Neimanis et al., 2010; Robinson et al., 2010; Ganas et al., 2014) or big size (Chavatte et al., 2019). Anderson et al. (2009) described also conjunctivitis, sinusitis and neurologic disease in mockingbirds (*Mimus polyglottos*). In finches, slight to marked thickened crop, and rarely, white to yellow masses in the oropharyngeal cavity were described (Forzán et al., 2010; Neimanis et al., 2010; Robinson et al., 2010; Ganas et al., 2014; Madani et al., 2015). Psittaciformes like budgerigars (*Melopsittacus undulatus*) or cockatiels (*Nymphicus hollandicus*) presented lesions described as abscesses in the oropharyngeal cavity, crop or thoracic/distal esophagus (Park, 2011). In the order Galliformes, Stockdale et al. (2015) described the case of a positive red-legged partridge (*Alectoris rufa*) with a caseous lesion at the proventriculus spreading to the liver. Finally, a toco toucan (*Ramphastos toco*), from the order Piciformes, was found dead of trichomonosis, presenting two caseous masses on the surface of the pharynx and esophagus (Ecco et al., 2012).

All this information about the presence of typical macroscopic lesions in the oropharyngeal cavity is very helpful in the diagnosis of the parasite, but there is little information about the dimensions, location or affected structures in order to establish an adecuate diagnosis and prognosis of the disease in different avian orders. To provide proper and quick veterinary care and treatment, data related to the severity of trichomonosis will be useful for veterinary practinioners.

## 2. Material and methods

### 2.1. Origin of the samples

A formal petition was submitted to the nine wildlife rehabilitation centres (WRCs) with the highest number of admissions in Spain to obtain clinical records from birds with lesions compatible with oropharyngeal trichomonosis. Five of them responded in a positive manner: the wildlife veterinary hospital of "Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat" (GREFA, Madrid, Spain), WRC of La Granja de El Saler (Valencia, Spain), WRC of La Alberca (Murcia, Spain), WRC of Alicante (Santa Faz, Alicante, Spain) and "Centro de Estudios de Rapaces Ibéricas" (CERI). Data on clinical cases with lesions were obtained from 2006 to 2017.

Furthermore, fourteen Bonelli's eagles (*Aquila fasciata*) were examined directly from nests for diagnosis of oropharyngeal trichomonosis in the context of conservation projects, ten from the European LIFE program (LIFE12 NAT/ES/000701-Integral recovery of Bonelli’s eagle population in Spain) in cooperation with GREFA, and four of them in collaboration with the Conselleria d'Agricultura, Medi Ambient, Canvi Climàtic i Desenvolupament Rural (Generalitat Valenciana).

## 2.2. Birds

A total of 94 clinical cases of birds presenting lesions compatible with trichomonosis were evaluated for the description of their lesions, 80 of those from birds of prey and the remaining 14 from Columbiformes. In the first group, animals belonged to 13 different species from three orders. Thirty-six of them were Accipitriformes of seven species: four goshawks (*Accipiter gentilis*), two Eurasian sparrowhawks (*Accipiter nisus*), 21 Bonelli's eagles, three booted eagles (*Aquila pennata*), three common buzzards (*Buteo buteo*), two marsh harriers (*Circus aeruginosus*) and one red kite (*Milvus milvus*). Fifteen were Strigiformes of four species: one long-eared owl (*Asio otus*), four eagle owls (*Bubo bubo*), seven tawny owls (*Strix aluco*), and three barn owls (*Tyto alba*). Twenty-nine were Falconiformes of two species: three peregrine falcons (*Falco peregrinus*) and 26 common kestrels (*Falco tinnunculus*). In the order Columbiformes, three different species were examined: five Eurasian collared doves (*Streptopelia decaocto*), five rock pigeons (*Columba livia*) and four wood pigeons (*C. palumbus*).

As a routine protocol in rehabilitation centres, animals showing lesions compatible with trichomonosis were treated with nitroimidazole drugs or underwent surgery to remove the lesions. Sixty-three of the examined animals died shortly after their admission at the centres or arrived dead. Some of the birds presented emaciation with severe lesions potentially impeding normal feeding. Since an unfavorable prognosis was determined, they were euthanized. The other 31 animals responded promptly after treatment for the flagellate infection.

## 2.3. Postmortem Examination

Carcasses were stored at 4-6ºC until necropsied as soon as possible (within 24 hours of dead), following the same routinary protocol (Stockdale et al., 2015). The body condition was evaluated by calculating the ratio of sternum musculature to body weight, as previously described by other authors (Harrison and Richie, 1995; Molina-López et al., 2015). When animals had solid rounded pectoral muscles, the body condition was categorized as "normal"; when the muscles were atrophied, with a prominent sternum, category was "low". External and internal organs were examined and the description of both clinical signs and lesions related to trichomonosis was registered.

## 2.4. Clinical cases and laboratory diagnosis techniques

In this study, clinical cases with gross lesions consistent with avian trichomonosis were considered when the following criteria appeared: dead or alive birds that harboured the parasite and had lesions compatible with trichomonosis at least in the oropharyngeal cavity, crop, eyes, liver or skull. Lesions of the crop detected by palpation and, in all cases, verified by necropsy. Laboratory diagnosis was performed at least by one of the following methods: wet-mount smear, culture or PCR and sequencing, as detailed below.

Samples for wet-mount smears and culture were taken with sterile cotton swabs from the upper digestive tract. Trophozoites with their characteristic size, morphology and motion were visualized under the microscope (400x) on direct wet-mount smear made from one swab. A second swab, previously moistened in the culture medium, was inoculated into 5 mL of pH 6.5 tryptose-yeast-maltose (TYM) medium (Martínez-Herrero et al., 2014). The culture was immediately incubated at 37 ºC and visually monitored with an inverted microscope every 24 h for 10 days to check the presence of the flagellates.

DNA extraction was carried out from swabs obtained from carcases using DNeasy Blood and Tissue Extraction Kit (QIAGEN, Valencia, California, USA), and positive (1oo µl of *T. gallinae* culture) and negative controls (sterile swab) were included in each batch. PCR and sequence analysis were performed using the ITS1/5.8S/ITS2 region (ITS). Oligonucleotide primers and thermo cycler temperature profiles were used according to Felleisen (1997). The primers employed were TFR1 (5’-TGCTTCAGCTCAGCGGGTCTTCC-3’) and TFR2 (5’-CGGTAGGTGAACCTGCCGTTGG-3’. The reaction was done in a final volume of 50 µL, containing 5 µL of 10 x buffer, 1.5 mM MgCl2, 2 mM dNTP, 2 µM of each primer, 2.5 IU Taq polymerase (MP, Thomas Scientific, Swedesboro, New Jersey, USA) and 5 µL of genomic DNA. The PCR protocol started with an initial step to activate the enzyme at 95ºC for 9 minutes, followed by 40 cycles of denaturation at 94ºC for 30 seconds, annealing at 66ºC for 30 seconds, extension at 72ºC for 30 seconds and a final extension step at 72ºC for 15 minutes. Electrophoresis was done in a 1.5% agarose gel stained with ethidium bromide (0.5 µL/mL) at 80V for 35 minutes. Ten µL of each sample was loaded and gels were observed under ultraviolet light. All reactions were carried out in a Gene Amp 2700 thermo cycler (Applied Biosystems, Foster City, California, USA). Results were observed under UV light in a transilluminator (Syngene, Cambridge, UK). PCR amplification products were purified with MinElute PCR Purification kit (QUIAGEN) and submitted for sequencing to the laboratories Sistemas Genómicos, S.A. (Paterna, Valencia, Spain) (Martínez-Herrero et al., 2014). Positive and negative controls (autoclaved water) were also included to ensure results and absence of contamination.

## 2.5. Pathological criteria for description of the lesions

Birds with gross lesions compatible with avian trichomonosis were classified into three different categories (mild, moderate and severe) attending to the following three criteria: the size of the lesions (or the sum of affected areas in case of multifocal distribution), the grade of depth and the location (Table 1). The tracheal opening or glottis was selected for the comparison of the size of the lesions using as a reference a specific anatomical structure. Regarding to the first criteria (size), lesions were classified as mild when they measured less than 50% of the tracheal opening; moderate when they measured between 50 and 100% of the tracheal opening, and severe when the measure was more than 100% of it. Attending the depth grade, lesions were considered mild when they were superficial, and the necrotic material came away easily, and with no underlying lesion; moderate in case of deep lesions, when the necrotic material was attached to the underlying mucosa, extending into the soft tissues, and severe in very deep lesions when the necrotic material was attached to the subjacent mucosa, extending into the deeper tissues (cartilage and/or bone). Finally, attending to the location, lesions were considered mild when they appeared in the choanal slit or the tip of the tongue and moderate if the location was the beak angle, the palate, the infundibular cleft or the eye. In case of lesions located close to the oropharyngeal opening like esophagus, crop, base of tongue, or to vital organs like skull, they were considered severe.

In general, a concordance between the three criteria was observed, but some cases showed discrepancies. Discordant cases were considered as moderate when at least one criterion was moderate and none severe, whilst severe cases were those with at least one criterion classified as severe. Cases with the highest discordance (one mild, one moderate and one severe criterion) were considered as moderate. Figure 1 details the anatomical regions of the oropharyngeal cavity that may have lesions.

### 2.6. Statistical analysis

We used chi square test ($\chi^2$) with R-3.2.4 software for Windows to asses the differences in mortality rate in the four different avian orders, considering the severity of the lesions. Differences were considered significant when *p* value was $< 0.05$.

## 3. Results

### 3.1. Necropsies

Three goshawks, two eagle owls and one tawny owl arrived dead to the rehabilitation centres and were necropsied (Table 2). All the birds presented dehydration and cachexia, as well as lesions consistent with trichomonosis at the oropharyngeal cavity. On the other hand, necropsies were also carried out on eleven Accipitriformes (all hawks, two booted eagles, one common buzzard, one marsh harrier and the red kite), ten Strigiformes (two eagle owls, five tawny owls and three barn owls), twenty-seven Falconiformes (one peregrine falcon and twenty-six common kestrels) and on the fourteen Columbiformes that died during admission to the centres or were euthanized. In all cases, dehydration and poor nutritional condition was observed, as well as clinical sings including swollen head or eyes, and wet feathers around the beak (Fig. 2). Three cases of goshawks with lesions of fibrinonecrotic material extended through the mucosa of the upper and lower regions of the oropharyngeal cavity were also infected by capillarid nematodes that were idientified according to the morphology of the eggs (Figs 3A and 3B).

## 3.2. Anatomical location of the lesions

Genotypes isolated from animals with lesions examined in this paper have been previously described (Sansano-Maestre et al., 2009; Martínez-Herrero et al., 2014; Sansano-Maestre et al., 2016).

In reference to the distribution of lesions, considering all clinical cases of dead and alive animals, most (70.2%) of the birds showed changes in multiple anatomical locations, whilst 29.8% presented a unique lesion. Multifocal distribution was more frequent in Falconiformes and Columbiformes than in Accipitriformes and Strigiformes (Figs. 4A and 4B).

Species from the order Accipitriformes had lesions located at the upper jaw (UJ) (including the palate, choanal slit and infundibular cleft), at the lower jaw (LJ) (including tongue), the eye, the skull, as well as the esophagus (Fig. 5). The same findings were observed in Falconiformes, although the crop was also invaded in several animals of this group (Table 2). Nocturnal birds of prey (order Strigiformes) presented lesions at the UJ, eye, and skull. Finally, in the case of order Columbiformes, lesions were located at the UJ, LJ, eye, esophagus, and also the crop. Thus, the oropharyngeal cavity area with the higher number of lesions was the UJ, with 71.3% of the birds affected (67/94). This anatomical region showed lesions in 61.1% (22/36) of the Accipitriformes, in 93.3% of Strigiformes, except in a tawny owl (14/15), in 10.3% of peregrine falcons (3/29) and in 51.7% (15/29) of common kestrels in the group of Falconiformes, and in 92.9% Columbiformes, except in a wood pigeon (13/14). Secondly, the LJ region accounted for 62.8% (59/94) of the birds. Of them, 21 were Accipitriformes (58.3%), 27 Falconiformes (93.1%) and 11 Columbiformes (78.6%). In 46.8% of the animals (44/94), both UJ and LJ regions were found to be affected. Most of them belonged to the group of Falconiformes (16/29; 55.2%), followed by Columbiformes (10/14; 71.4%), while Accipitriformes and Strigiformes showed this distribution less frequently (7/36; 19.4% and 1/15; 6.7%, respectively). The esophagus was also involved in 12.8% of the cases (12/94), one of which was a booted eagle (Accipitriformes) (1/36; 2.8%). In one wood pigeon (Columbiformes), and in ten common kestrels (Falconiformes) (10/29; 34.5%), lesions extended to the crop. Next, the eye, considering animals which presented with conjunctivis defined as external swelling of the eye lids, with ocular discharge, or a yellow mass invading the orbital area was determined in 9.6% of the cases (9/94) (Fig. 2). Of them, three were goshawks, one sparrowhawk, one tawny owl, two peregirne falcons one common krestel and one wood pigeon. A barn owl and a marsh harrier had also the skull affected (2/94; 2.1%). Finally, a common kestrel and a rock pigeon presented caseonecrotic lesions compatible with trichomonosis in the liver, besides the ones observed at the oropharyngeal cavity (n = 2/94; 2.1%), but laboratory tests to evaluate the presence of *Trichomonas* at the organ were not performed in these cases.

### 3.3. Classification and prognosis

Considering the three criteria for performing the classification, most animals (17/94; 18.1%) had lesions measuring between 0 and 50% (mild) of the tracheal opening. In twelve of them (12.8%), the size was between 50-100% (moderate). Extended lesions (severe) were found in 65 of cases (69.1%), when it occupied more than 100% of the tracheal opening. The second criteria was the depth grade of lesions. Some animals (19/94; 20.2%) presented superficial lesions, with no visible alterations in the lower tissue (mild). In ten birds (10.6%), deep lesions, with the swollen lower tissue were observed (moderate). In the most serious cases (65/94; 69.2%), lesions were located very deep, usually accompained by inflammation of the neck or the face (severe lesions). Finally, attending to the location, a low number of animals (11/94; 11.7%) showed lesions present in areas far from the oropharyngeal opening, such as the choanal slit or the tip of the tongue. Lesions at the beak angle, palate, infundibular cleft, or eye were observed in 24 animals (25.5%). In most cases (59/94; 62.8%), esophagus, crop, or the base of the tongue were the locations of the lesions, so the ability to swallow was compromised and the prognosis was worse. The same was true for the barn owl, although in this case the necrotic lesion caused osteolysis of the skull and extended into the brain.

Considering the three criteria for pathogenicity, 10.6% of the cases (10/94) showed lesions of mild grade (Fig. 6A). Moderate grade was found in 18.1% of the birds (17/94) and severe grade in 71.3% of the specimens (67/94) (Figs. 6B, 7A, 7B, 8A, and

8B). In the group of raptors, lesions of mild, moderate and severe grade were found, whereas columbiformes presented exclusively moderate and severe lesions. Mild grade was observed only in eagles, nine Bonelli´s eagles and one booted eagle.

In the order Accipitriformes, lesions of the three gradations were observed in the seven species (Table 2). Nine Bonelli's eagles (of which six were examined at the nests) and one booted eagle presented mild lesions; two goshawks, eight Bonelli's eagles, one marsh harrier and the red kite showed lesions moderate lesions and the four common buzzards, the two Eurasian sparrowhawks, two goshawks, two booted eagles, four Bonelli's eagles and one marsh harrier had severe lesions. In the group of Falconiformes, except for two common kestrels with moderate lesions, all the animals presented severe lesions. In the order Strigiformes, most of the animals had severe lesions, whilst moderate lesions were found just at the long-eared owl and in one tawny owl. Finally, in the order Columbiformes, only one moderate lesion was observed in a wood pigeon. The rest of the birds in this group showed severe lesions and died shortly after admission at the rehabilitation centres.

In lesions of mild grade, the prognosis was optimal, as they had the smallest dimensions, inflammation was absent or slight, and the tracheal opening was not compromised. The recovery rate in this group of animals was 100% after treatment or surgery, since all the animals showing this grade survived (Fig. 9). Moderate lesions had a doubtful prognosis, since almost half of the birds in this group finally died (7/17; 41.2%). In many cases lesions at this stage already had a multifocal distribution. Finally, euthanasia or death was the main outcome in birds with severe lesions. Prognosis of these birds was significantly poorer than in those with mild and moderate lesions ($p < 0.01$). Thus, 82.1% (55/67) birds died likely as a result of the protozoal infection.

The mortality rates of the animals with the severe form were significantly higher in the groups of Falconiformes and Columbiformes than in Strigiformes or Accipitriformes ($p < 0.01$). In the first one, 92.6% (25/27) of the animals with severe lesions died, in addition to the two animals that had moderate lesions. In the second group, all the animals died, including the only wood pigeon harboring moderate lesions. Most of the nocturnal raptors died (66.7%, 10/15), as well as 30.6% (11/36) of Accipitriformes (Fig. 10).

## 4. Discussion

Faced with the severity of avian trichomonosis, it is vital to perform a quick routine examination when treating domestic and wild birds, since the stress of management at clinics and rehabilitation centres favours the parasite overgrowth (Stenkat et al., 2013).

Thus, in this review of cases, a macroscopical evaluation of gross lesions at the oropharyngeal cavity compatible with avian trichomonosis infection was performed and a clinical categorization of affected birds into three grades was made, according to their severity.

The genotypes of *Trichomonas* identified in the studied clinical cases were previously described. A direct relation between the genotype and the preference of the prey items in their diet was found in animals with gross lesions, being genotype ITS-OBT-Tg-1GenBankacc.n. EU881911 identified in all animals with severe lesions (Sansano-Maestre et al., 2009; Martínez-Herrero et al., 2014).

All necropsied animals presented a low body condition, to a greater or lesser degree, potentially due to the hardship of feeding originated by the lesions. Molina et al. (2015) found that a low body condition was the main prognostic factor related to mortality in wild raptors admitted to rehabilitation centres, regardless of their age. Infected birds are more susceptible to many disease agents, and in case of prey items as pigeons, turtle doves or other small species, they are also more accessible to raptors. Thus, endangered prey species become exposed to trichomonosis, increasing the hazard of infection in nestlings. Gross lesions usually appear in non-adapted hosts, in a more severe form in chicks (Real et al., 2000; Höfle et al., 2004; Villanúa et al., 2006; Martínez-Herrero et al., 2014; Rogers et al., 2016).

In the orders Accipitriformes and Falconiformes, the subset of birds examined showed anatomical predilection sites already described by several authors in diurnal birds of prey (Real et al., 2000; Samour and Naldo, 2003; Krone et al., 2005; Martínez-Herrero et al., 2019). In summary, the distribution of the lesions was similar at the UJ and the LJ.

In Falconiformes, common kestrels showed lesions of greater size and poorer prognosis. This might be explained because their diet is not strictly ornitophagous, so they are not immunologically adapted to the parasite (Amin *et al*., 2014). However, it seems to be an increase in the number of individuals of this species infected with trichomonosis in recent years, probably due to a change in their diet, especially in urban and peri-urban areas, where access to highly parasitized Columbiformes is elevated (Martínez Herrero et al., 2014).

In the present work, lesions were described and evaluated from species with scarce reports in literature. For instance, booted eagles, common buzzards, a marsh harrier and a red kite had gross moderate and severe lesions and the presence of trophozoites of *Trichomonas* spp. was confirmed by culture. Avian trichomonosis is not commonly reported in this species, presumably since columbiforms are infrequent in their diet.

Interestingly, in the order Strigiformes all birds displayed gross lesions that involved the upper region of the oropharyngeal cavity (palate, choanal slit, infundibular cleft), eye and/or tissues of the skull basis. The same anatomical regions have been described to be affected with gross and extense lesions in other nocturnal birds of prey (Jessup, 1980; Pokras et al., 1993; Sansano-Maestre et al., 2009; Ecco et al., 2012). In 1980, Jessup reported three cases of avian trichomonosis in great horned owls (*Bubo virginianus*) that reached the soft tissues and bones of the skull base. We had observed a similar trend for the parasite locations in species belonging to the same order of birds, which could be explained by their anatomical and physiologycal characteristis. The animals in which a necropsy was performed did not show further internal lesions of avian trichomonosis. It could be possible that the absence of crop in nocturnal birds of prey implies that the parasite will primarily start its multiplication at the oropharyngeal cavity, extending to the palate and bones of the skull, instead of esophagus (Duke, 1997). Certainly, the specific tissue pH would be another factor that influences the areas where the flagellated is able to propagate. In fledgling and breeding Cooper´s Hawks, with an acidic oral condition, the parasite showed less viability than in nestlings, with an oral pH close to the optimal for the growing of *Trichomonas* spp. (Urban et al., 2015; Taylor et al., 2019). There is no information on the pH in the esophagus of Strigiformes, but it could be of interest to investigate if there are differences between the crop milieu of other raptors.

Lesions in reservoir hosts of the parasite (order Columbiformes) have been previously described by other authors at the oropharyngeal cavity and upper gastrointestinal tract (esophagus, crop) and internal organs (liver, lungs, pancreas, etc.). In this study, lesions were detected in some of these locations as well, mainly in the oropharyngeal cavity but also in the crop, esophagus and orbital sinuses. One wood pigeon had necrotic lesions in the liver, like those described by other authors in the same species (Stenkat et al., 2013), as well as in a band-tailed pigeon (Girard et al., 2014) and in a European turtle dove (Stockedale et al., 2015), although we could not confirm the presence of the protozoa in the hepatic tissue. The Eurasian collared dove, a species that has recently increased its population size and range in western Europe and America, was found with gross lesions of avian trichomonosis in the oropharyngeal cavity as described for other columbiform hosts, such as the rock pigeon (Pérez-Mesa et al., 1961) or the wood pigeon (Höfle et al., 2004). This finding agrees with the fact that this species is not only a reservoir of the parasite, but it is also affected by pathogenic strains of the parasite, as previous authors reported from an outbreak in the Caribbean (Stimmelmayr et al., 2012).

Most of the studied specimens presented severe lesions, which is expected, considering that they came from rehabilitation centres, probably weakened due to trichomonosis and/or other health problems. This fact may not reflect the real situation in nature, and the animals could respond in a better way to the disease, depending on their health status. On the other hand, many birds with mild lesions belonged to the order Accipitriformes, especially Bonelli´s eagles. This can be explained by the fact that some of them were examined directly at nests, where chicks are usually diagnosed at the beginning of the disease.

It has been observed that animals with severe lesions have a clear poor prognosis, especially in the group of Falconiformes, where only two peregrine falcons finally survived. Common kestrels had a worse outcome, with large lesions almost always located in the lower jaw, esophagus and crop. In addition, Columbiformes showed a death rate of 100%. As usual hosts for the protozoa, columbiformes are parasitized without presenting lesions on numerous occasions (Höfle et al, 2004; Sansano-Maestre et al., 2009). Nevertheless, the high mortality rate found in this study is probably because the animals with lesions are those that reach the recovery centres.

The description of the lesions includes information on the prognosis of the disease. Namely, in animals with mild lesions, treatment with nitroimidazoles is recommended as well as the cleaning of the granulomas. Moderate lesions require treatment to be administered as soon as possible as they already have a multifocal distribution, indicative of a rapid spread and multiplication rate of trophozoites through the mucosal layer of the oropharyngeal cavity. Surgery is also necessary to remove the most superficial caseous plaques in this group. Finally, severe lesions implie an urgent surgery followed by antiprotozoal therapy, due to extensive lesions with a general poor body condition and a high risk of death. In common krestels, pigeons and doves at this grade, euthanasia is recommended taking into account the nonexistent recovery of any of these animals.

The importance of confirming the diagnosis by direct extension, culture or PCR, as it has been performed in this study, is remarkable. There are other infectious processes, such as candidiosis, capilariasis or salmonellosis, whose lesions are very similar to those produced by *Trichomonas* with mild and moderate lesions, and it is not unusual to find co-morbidity in some cases (Alkharigy et al., 2018), although salmonellosis and hypovitaminosis A are not frequently reported in wild birds. In fact, three of the goshawks were found to be infected with *Capillaria* spp., a parasite that is not uncommon in these birds (Childs-Stanford et al., 2018). Furthermore, it is essential to combine a method of rapid diagnosis in animals with lesions with a routine sampling in animals that do not show lesions, since positive with no lesions have been described, even when infected with pathogenic strains, especially in Columbiformes, their usual hosts (Vilanúa et al., 2006; Stockdale et al., 2015; Zu Ermgassen et al., 2016). Thus, treatment would be also recommended in these animals that can act as a reservoir for the disease (Bunbury, 2011).

*Trichomonas* was isolated in all cases, although we cannot rule out the presence of other infectious diseases in these animals, or even other debilitating processes, such as toxics or immune depression. However, for mild and moderate lesions, the positive response to nitroimidazole treatment is an indication of the involvement of the parasite in the development of lesions (*Candida* and virures do not respond in this way). On the other hand, severe lesions described in the oropharyngeal cavity are considered pathognomonic for *Trichomonas*. They were in the specific location (oropharyngeal cavity), typical caseonecrotic nodules and with the causative agent having been isolated. In other common avian diseases like candidiasis, we would expect to see more superficial lesions (not forming deeply attached caseous nodules) extending through the esophagus and affecting the crop. In any case, whether if *Trichomonas* is the only agent, or if there is a co-infection, the end of animals with severe lesions is almost always the death.

This paper can be used as a helping tool for the evaluation, prognosis and understanding the pathologic and epidemiological information about avian trichomonosis among veterinary related professionals. Additionally, a deeper knowledge about avian diseases and differential diagnosis of death in birds can also help in establishing proper and quick treatments as well as in making decisions.

## 5. Conclusion

Lesions of oropharyngeal trichomonosis present different anatomical locations regarding on the avian order. In this paper a revision of lesions was done attending to their location, as well as size and depth, in order to help in the management of the disease. Animals with mild and moderate lesions have a favorable resolution when treated, while the presence of severe lesions implies dead or euthanasia in most cases.

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**Table captions**

Table 1. Type of lesions in oropharyngeal trichomonosis considering the following criteria: size in relation with the tracheal opening, depth and anatomical location.

Table 2. Grades, of the lesions according to the following criteria: location, depth and size in relation to the tracheal opening. Affected anatomical regions include: upper jaw

(UJ, including palate, choanal slit and infundibular cleft), lower jaw (LJ, including the tongue), eye, skull and esophagus. The evolution of the birds analyzed in the study is included.

**Figure captions**

Figure 1. Anatomical regions of the oropharyngeal cavity of a nestling of Bonelli's eagle (*Aquila fasciata*)

Figure 2. Swollen head and orbital area due to trichomonosis in a common krestel, (case 74, lateral view)

Figure 3. Lesions due to *T. gallinae* and capillarids in a goshawk, (A, case 1, frontal view) (B, capillarid eggs from a smear)

Figure 4. Multifocal severe lesions in a marsh harrier (A, case 35, frontal view of the oropharyngeal cavity) and moderate lesions in a long-eared owl due to *T. gallinae* infection (B, case 37, frontal view of the oropharyngeal cavity)

Figure 5. Distribution and grade of extension of the lesions in the different avian orders included in the study. The size of the black circles indicates the percentage of animals with trichomonosis that showed lesions at the referred anatomical location.

Figure 6. Mild (A, case 24, frontal view of the oropharyngeal cavity, lower jaw) and moderate lesions (B, case 7, frontal view of the oropharyngeal cavity, upper jaw) in Bonelli's eagles due to *T. gallinae*

Figure 7. Severe lesions of trichomonosis in common kestrels (A, case 74, frontal view of the oropharyngeal cavity; B, case 73, frontal view of the oropharyngeal cavity)

Figure 8. Severe lesions in a Eurasian collared dove (A, case 81, lateral view) and in a rock pigeon (B, case 88, lateral view) due to *T. gallinae* infection

Figure 9. Mortality rate (%) in animals with mild, moderate and severe lesions due to *T. gallinae* infection

Figure 10. Mortality rate in animals with mild, moderate and severe lesions due to trichomonosis for each order