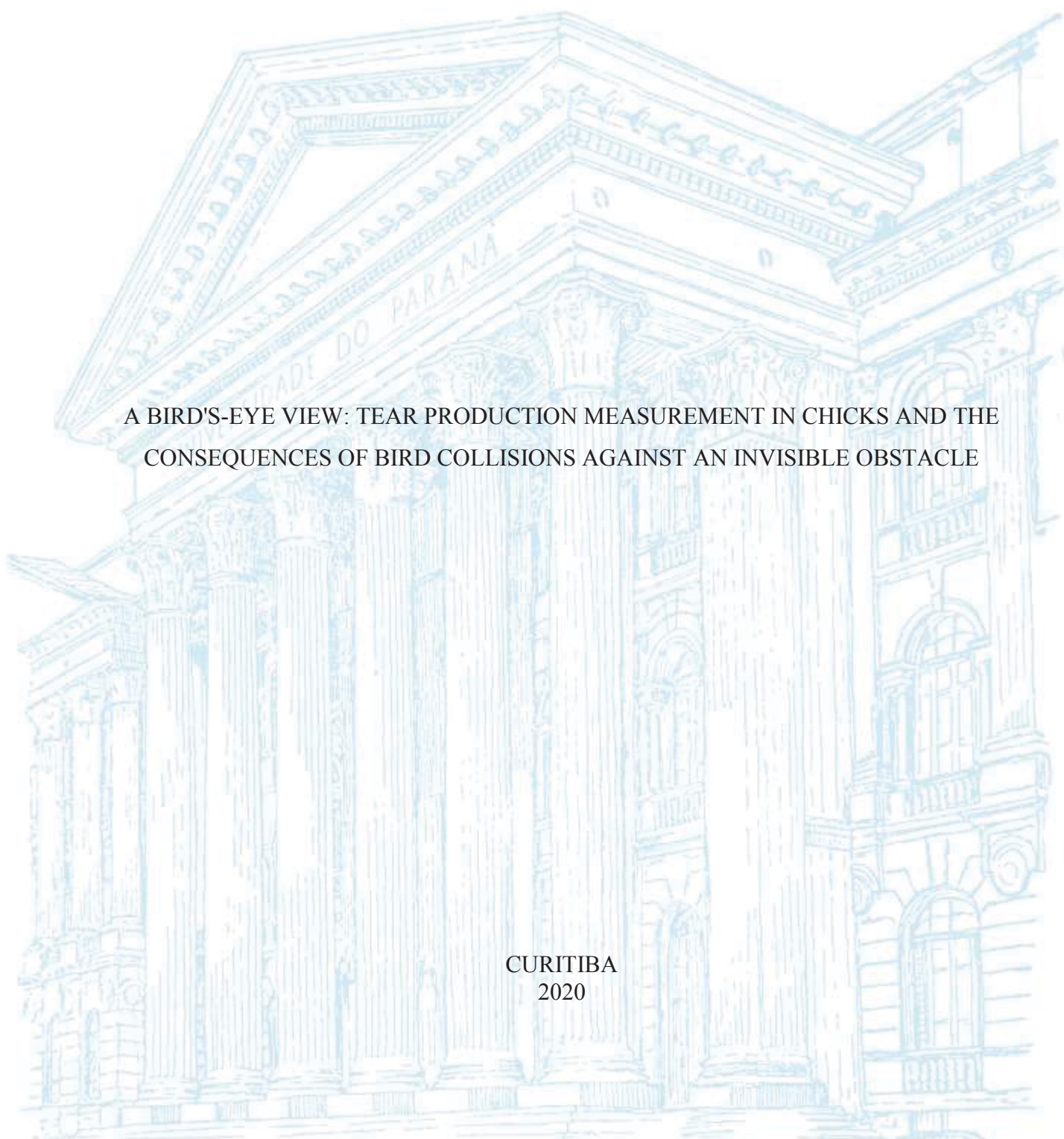


UNIVERSIDADE FEDERAL DO PARANÁ

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A BIRD'S-EYE VIEW: TEAR PRODUCTION MEASUREMENT IN CHICKS AND THE
CONSEQUENCES OF BIRD COLLISIONS AGAINST AN INVISIBLE OBSTACLE

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2020



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CONSEQUENCES OF BIRD COLLISIONS AGAINST AN INVISIBLE OBSTACLE

Tese apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, do Setor de Ciências Agrárias, da Universidade Federal do Paraná, como requisito parcial para obtenção de título de Doutora em Ciências Veterinárias.

Orientador: Professor Ph.D. Fabiano Montiani-Ferreira

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **GABRIELLE ADAD FORNAZARI** intitulada: **A BIRD'S-EYE VIEW; TEAR PRODUCTION MEASUREMENT IN CHICKS AND THE CONSEQUENCES OF BIRD COLLISIONS AGAINST AN INVISIBLE OBSTACLE**, sob orientação do Prof. Dr. **FABIANO MONTIANI FERREIRA**, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua **APROVAÇÃO** no rito de defesa. A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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A ideia de uma pesquisa acadêmica nasce fundamentalmente da interação entre orientador e orientado. Contudo, apenas o esforço individual do último é que realmente a faz crescer. Além disso, só por meio do apoio recebido dos coautores e colaboradores é que a pesquisa pode se aperfeiçoar. No caso específico desta tese, os Professores Daniel Klem, Alex Maiorka, Tilde Froes, Elizabeth Santin, Rogério Lange, e os colegas André Saldanha, Josiane Carla Panisson e Bret A. Moore foram aqueles que fizeram exatamente isso: auxiliaram sobremaneira para que todas as investigações fossem melhoradas e concluídas. Agradeço o trabalho da PRPPG-SIGA-UFPR pelo auxílio prestado na confecção do webstite. Agradeço ainda aos meus queridos amigos e, principalmente, meus familiares que ao longo desta longa jornada me motivaram e me apoiaram de várias formas, as vezes até indiretamente cuidando do meu filho, fazendo com que esta fosse uma das fases mais produtivas da minha vida.

Epígrafe: *“Tame birds sing of freedom. Wild birds fly.” John Lennon*

RESUMO

A presente tese consiste de dois capítulos, cada qual, composto por um artigo científico distinto. Ambos englobam temas relacionados direta ou indiretamente com visão de aves, sendo relevantes para a Oftalmologia Veterinária, bem como para a Medicina Zoológica. Todos os estudos foram conduzidos pela autora, orientador e colaboradores do Laboratório de Oftalmologia Comparada (LABOCO) da Universidade Federal do Paraná (UFPR). O primeiro capítulo é composto por um artigo científico denominado “*Schirmer's I, modified Schirmer's I, phenol red thread, and paper point tests: a comparative study for tear production measurement techniques in broiler chicks (Gallus gallus domesticus)*” e compreende uma comparação de diferentes métodos para mensuração lacrimal em galinhas. Este capítulo fornece informações inéditas, contribuindo para o conhecimento da oftalmologia de um animal de produção comum, que eventualmente também é atendido em clínicas veterinárias. O estabelecimento de parâmetros normais de testes oftálmicos pode auxiliar médicos veterinários no diagnóstico de doenças oculares no futuro. O segundo capítulo é composto por outro artigo científico, este denominado “*Window Collisions by Birds in The South of Brazil: Epidemiologic Factors, Radiographic and Necroscopy Findings*” descreve as lesões corporais oriundas dos choques de aves em vidros, principalmente sob o ponto de vista de exames necroscópicos e radiográficos. Foram relatados acidentes principalmente durante a primavera, em machos com gônadas ativas, em um total de 34 espécies diferentes de aves em 22 famílias, sendo as três espécies mais prevalentes: sabiá-laranjeira (*Turdus rufiventris*), avoante (*Zenaida auriculata*) e rolinha-roxa (*Columbina talpacoti*). As espécies migratórias foram menos frequentemente acidentadas. A lesão radiográfica mais comumente observada foi a perda de definição dos órgãos da cavidade celomática, sinal sugestivo de hemorragia. Os achados de necropsia sugeriram que o trauma cranioencefálico é a principal *causa mortis* das aves que sofrem este tipo de acidente.

PALAVRAS-CHAVE: Oftalmologia. Visão. Produção Lacrimal. Aves. Trauma Cranioencefálico. Vidro.

ABSTRACT

The present thesis consists of two chapters, each prepared of different scientific articles. Such chapters cover topics related directly or indirectly to bird vision and are relevant to Veterinary Ophthalmology, as well as to Zoological Medicine. The main author, the advisor and collaborators of the Comparative Ophthalmology Laboratory (LABOCO) at the Federal University of Paraná (UFPR) conducted all studies. The first chapter consists of a scientific article called “Schirmer's I, modified Schirmer's I, phenol red thread, and paper point tests: a comparative study for tear production measurement techniques in broiler chicks (*Gallus gallus domesticus*)” and consists of a comparison of different methods for tear measurement in this specie. This chapter provides original information, contributing to the knowledge of ophthalmology of a common production animal, which eventually become a patient in veterinary clinics as well. The establishment of normal ophthalmic test parameters can assist veterinarians in diagnosing eye diseases in the future. The second chapter consists of another investigation, this one called “Window Collisions by Birds in The South of Brazil: Epidemiologic Factors, Radiographic and Necroscopy Findings”, which describes the injuries resulting from glass collisions, mainly from the point of view of necroscopic and radiographic exams. Most accidents have been reported during spring, in males with active gonads, in a total of 34 different bird species belonging to 22 families, the three most prevalent species being: Rufous-bellied thrush (*Turdus rufiventris*); Eared dove (*Zenaida auriculata*) and Ruddy ground dove (*Columbina talpacoti*). Migratory species were less frequently victims of this type of accident. The most common radiographic lesion observed was loss of definition of many internal organs in the coelomic cavity, suggestive of hemorrhage. The necropsy findings suggested that traumatic brain injury is the main cause of death for birds suffering from this type of accident.

KEY-WORDS: Ophthalmology. Vision. Lacrimal Production. Poultry. Cranioencephalic Trauma. Glass.

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1. GENERAL INTRODUCTION

The most important sensory modality for birds is vision, since an efficient eyesight is indispensable for performing a safe flight it also is essential to identify potential mates, find food or hunt, and to quickly identify and escape from predators (Güntürkün 1998, Jones et al. 2007). The evolution of the avian vision occurred in parallel to that of modern primates, with unique adaptations that greatly improved its visual acuity, even beyond that of humans. Bird eyes in general are large in relation to the body and head size (especially). In birds, the eye occupies approximately 50% or more of the cranial volume of the skull, whereas in humans, the eye occupies less than 5% of the skull's volume (Waldvogel 1990). The eyes of raptors, for instance, are about the same size of human eyes or even larger. Likewise, the eyes of the ostrich (*Struthio camelus*) are about twice as large as those of humans (King and McLelland, 1984; Samuelson, 1991; Güntürkün 1998). Due to their large size, the avian eye globe is tightly "packed" into a large bony orbit, making its eye movements very limited (Güntürkün 1998). Consequently, when a bird wants to move its eyes, it also moves its head in the same direction. The eyes and orbits of most birds are positioned rather laterally, which provides great monocular visual fields and favors peripheral vision. However, in some species, especially raptors, a more rostral position of their globes creates a wide binocular visual field, typical of predators, which enables accurate judgement of distances. This feature is potentiated by and the presence of elongated globes (called tubular eyes) in some nocturnal species, like owls. In addition to the two eyelids usually found in most vertebrates, the birds' cornea is protected and lubricated by a semi-transparent conjunctival fold called third eyelid. The birds' cornea possess a Bowman's layer, an acellular, superficial limiting lamina underneath the corneal epithelium, just as humans' do. Its ocular internal anatomy follows a standard vertebrate pattern. Some features are somewhat similar to that of reptiles, with ciliary muscles that can adjust the shape of the lens (accommodation) rapidly and to a greater extent than that of mammals. In the same way that reptiles did, birds developed specializations inside the scleral tissue, a ring of ossicles that surrounds the eyeball, near the area of the lens, and a hyaline cartilage cup in the caudal aspect of the globe. This cartilage cup extends rostrally almost to the point of reaching the scleral ossicles. The number of scleral ossicles can range from 10 to 18, but most avian species have 14 to 15 (King and McLelland, 1984; Samuelson, 1991). These scleral features help to support and partially protect the large and exposed eye globes inside its large orbits. Iridal muscles are striated in birds. Thus, a neuromuscular blocking agent, for example, vecuronium, is necessary to initiate mydriasis and facilitate examination of the retina, not the parasympatholytic agents

routinely applied for the same purpose in small animals. Investigations using various combinations of mydriatic agents used in avian species have been reported (Mikaelian et al., 1994; Barsotti et al. 2012). Large eyes possess large pupils that allows more light to enter the posterior segment. More photons entering the eye also allow birds to see in lower light conditions, due to the greater retinal stimulation. Nonetheless, birds have a unique structure in the posterior segment called the pecten oculi. Most bird species (with a few exceptions) possess a fovea in their retinas, just as humans do. Unusually, the chicken possesses no fovea (Morris, 1982). Yet, some birds even have two foveae in each eye (Moroney and Pettigrew, 1987; Coimbra, 2014; Tyrrell and Fernandez-Juricic, 2017). The peregrine falcon (*Falco peregrinus*) is one example of a bird with two foveae. Acuity tests suggest that the peregrine falcon sees up to five times more detail than the human eye (Parker, 2016). Birds in general have a high density of photoreceptors with retinas that are rich in cones. Birds have a variety of cones cells, which are more complex than those of mammals are. The inner segment contains a clear or a carotenoid-colored oil droplet beside the base of the outer segment, which filters light before it can reach the visual pigments. The function of these oil droplets is not completely known, but they might may act as tiny lenses, filtering out potentially harmful wavelengths of light or they may enhance visual contrast. Besides single cones, birds also have double cones (two closely contiguous cones). Yet again, their role is not fully understood. Nocturnal species, however, have low numbers of cones but a high density of rod photoreceptors, which function well in poor light (Tyrrell et al. 2019)..

The chicken (*Gallus gallus domesticus*) is one of the most common and widespread domestic animals in the world, with a population of 23.7 billion as of 2018 (Statista 2018). As the most numerous bird on the planet, the chicken is unique due to a biology that was heavily shaped by humans. Chicken-meat consumption is growing faster than any other meat and soon it will probably surpass pork meat consumption worldwide. The biomass of humans and domesticated animals, combined, now outweighs that of all wild vertebrates on earth (Bennett et al. 2018). When future paleontologists examine our civilization, they will probably see bones of chickens amongst tin cans, glass bottles and bits of material that were once plastic. Thus, the modern chickens are a symbol of the impact of humans changing the world's biosphere (Bennett et al. 2018). In other words, humanity will probably be remembered for its chickens. Despite his species' popularity, before the work that constitutes the first chapter of this thesis, no studies measuring the normal tear production in chicks and adult chickens (*Gallus gallus*) were previously available in the scientific literature. In addition, studies evaluating age-related changes in tear production were also absent. Infectious diseases and the environment, especially

excessive concentrations of ammonia, can cause respiratory and ocular disease often accompanied by an increased lacrimal production in chickens. Thus, this ophthalmic investigation, although somewhat basic, was remarkably original and important for the field of veterinary ophthalmology and poultry science.

Why do birds that are a group of animals well known for having excellent eyesight recurrently collide with glass windows? For humans this sounds like a clear indication of poor eyesight. Yet, humans believe they can always see glass but actually, they do not. Instead, humans learned the “concept” of the existence of a solid, transparent/reflective material called glass from experience, early in life (Gibson and Walk, April 1960). Visual cues like mullions, window frames and reflected images help us to guess where to “expect” glass and, yet, people still hit glass often. Thus, not understanding glass as a physical/public concept is the real problem for birds. For birds glass windows are worse than invisible, they are treacherous. Glass virtually is a “bird trap”. By reflecting foliage or sky, they look like inviting places to fly into and not to avoid. As the number of windows is so great, their death toll is huge. Bird-window collisions are a major cause of bird mortality in the world. Just in the USA it is estimated that anywhere from 100 million to 1 billion bird deaths per year occur due to window strikes (Klem 1990). The second chapter of this thesis is the first investigation of bird window collisions performed in the south of Brazil bringing radiographic and necroscopic data. The investigation besides confirming evidences previously found in the literature, was responsible for reporting several original findings about this topic, such as the main radiographic features (cranial, extra-cranial fractures and loss of coelomic definition) encountered in this type of accident, as well as suggesting that cranioencephalic trauma and coelomic cavity hemorrhage, were the two most probable causes of death in the majority of these cases.

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2. SCHIRMER'S I, MODIFIED SCHIRMER'S I, PHENOL RED THREAD AND PAPER POINT TESTS: A COMPARATIVE STUDY FOR TEAR PRODUCTION MEASUREMENT TECHNIQUES IN BROILER CHICKS (*Gallus gallus domesticus*)

2.1 ABSTRACT

Problems with indoor husbandry and immunological challenges are some of the stressors that broilers may face which can affect production. Infectious diseases can compound the effects of excessive concentrations of ammonia causing respiratory and ocular inflammation and increased lacrimal production in broiler chickens. The objectives of this investigation were to establish normal values of tear production in chicks and compare the practicality of the different methods. Palpebral fissure length (PFL) and tear production were evaluated in Cobb 500 chicks at five-day-old and then at 42 days of age by the following methods: modified Schirmer's test I (mSTT1), phenol red cotton thread tear test (PRCTTT), and standardized endodontic absorbent paper point test (EAPPTT). Ten chicks at the age of 5 days and 42 days were evaluated for each method. In addition, at 42 days, when the palpebral fissure permitted, a regular-sized Schirmer tear test I (STT1) was performed in another group of 10 chickens. Overall, PFL and the secretion of the aqueous phase of the tear film increased with age. Mean mSTT1 results were 5.00 ± 1.83 mm and 10.45 ± 2.58 mm for 5 and 42 day old chicks, respectively. Mean PRCTTT results were 12.37 ± 1.80 mm and 25.58 ± 4.8 mm for five and 42 day old chicks, respectively. Mean EAPPTT results were 7.13 ± 0.72 mm and 12.03 ± 0.92 mm for five and 42 day old chicks, respectively. Mean STT1 for 42 day-old chicks was 11.40 ± 2.60 mm. Results obtained with endodontic paper points showed the lowest coefficient of variation and investigators found the test easier to perform. These values reported for broilers at different ages may be applied in poultry ophthalmology to help the diagnosis of lacrimal production disorders and to detect early disease symptoms that could cause economic losses in poultry production.

Key-words: Chick. Tear production. Normal parameters. Ophthalmology.

2.2 INTRODUCTION

According to the United Nations, world population is expected to reach 9.8 billion in 2050 (UN, 2017). Improving poultry health is a key component in the prevention of a food shortage (Pimentel, 2004). Active prevention and promotion of health in poultry production includes the diagnosis of disease at an early stage before an outbreak occurs (Gyles, 1989; Pimentel, 2004). Some of these diseases causes large economic losses and manifest an increase of lacrimal production due to inflammation of ocular and respiratory tissues. Examples of such diseases are Newcastle and Marek's diseases, reoviruses, salmonellosis, and aspergillosis, as well as poor management conditions that promote excessive ammonia production including poor ventilation (Calnek, 2001; Feitas Neto et al., 2007; Zande and Kuhn, 2007; Arné et al., 2011; Maliselo and Nkonde, 2015; Miles et al., 2006).

Three eye structures are responsible to produce the complex composition of the lacrimal film that covers the entire ocular surface. These structures are called: goblet cells, Harderian gland (also known as the gland of the nictitating membrane) and lacrimal glands. Goblet cells produce the mucine layer which is responsible to establish the lacrimal film, while Harderian and lacrimal glands produce the aqueous layer which is responsible to lubricate and nourish the eye surface (Kern and Colitz, 2013). Meibomian glands which are modified sebaceous glands have not been described in the lids of any avian species (Murphy, 1990). The aqueous fraction of the lacrimal film can be measured by simple and quick methods in the majority of domestic animals. In birds, however, the small size of the eye makes this challenging (Lange et al., 2012; Lange et al., 2014; Lima et al. 2015; Saleh et al., 2006). Schirmer tear test 1 (STT1) is the most commonly used method for lacrimal production measurement in humans and several species of animals. The test uses a standard millimeter absorbent paper strip. The tip is folded and positioned in the lower fornix for one minute (Lima et al. 2015). Because of its relative large width (5 mm), the test cannot be used in species with small palpebral fissures. There are three

alternative methods: modified Schirmer tear test 1 (mSTT1), phenol red cotton thread tear test (PRCTTT), and the endodontic absorbent paper points tear test (EAPPTT) (Silva EG; Lange et al., 2012; Lange et al., 2014; Lima et al. 2015; Saleh et al., 2006).

Many poultry diseases have the potential to affect lacrimal production. Standardization of normal tear test values can help in the early diagnosis of some of these ocular diseases in this species. To the authors' knowledge, however, no studies measuring tear production in chicks or adult chickens have been performed. In addition, studies evaluating age-related changes in tear production in chicks are also absent. Therefore, the purpose of this study was to establish normal tear production values for broilers at different ages. Additionally, three different techniques to quantify tear secretion in chicks were performed and compared.

2.3 MATERIALS AND METHODS

This study was approved by the institutional animal care and use committee and followed the guidelines of the Association for Research in Vision and Ophthalmology (ARVO)'s Statement for the Use of Animals in Ophthalmic and Visual Research. Forty chicks (Cobb 500) without signs of ocular or systemic diseases were used. The birds were reared in an experimental poultry house in wood shavings litter and kept at a comfortable temperature according to their age (1st week - 30°C, 2nd week - 26°C, 3rd week - 22°C, 4th to 6th week - 20°C) with water and feed *ad libitum*. The mashed diet was based on corn and soymeal following Brazilian nutritional recommendations for poultry (Rostagno, 2011).

2.3.1 Animals, ophthalmic examination screening, and environmental conditions

A total of 40 male chicks (Cobb strain) or 80 eyes were analyzed. Three groups of 10 chicks (30 eyes) at the age of 5 days then at 42 days, plus yet another 10 chickens (10 eyes) at

42 days of age. The animals were kept in an opened house system with a 10 cm pine shavings-litter. Wet litter was turned or replaced when excessively wet every 3 days during the experiment. All animals were evaluated with a slit lamp biomicroscope (SL-17, KOWA, Japan) to confirm the absence of ocular abnormalities.

The animals were tested at 5 and 42 days of age. Mean humidity was 60% and temperature was 30°C when the animals were tested at five days of age. At 42 days of age, mean humidity was 65% and temperature 20°C (Color Weather Station, AcuRite, Lake Geneva, WI, USA).

Atmospheric ammonia levels at chick eye height was measured and annotated using a calibrated automated toxic gas detector with a range 1 to 1000 ppm (BlockGas model PB-120, São Paulo-SP). Mean ammonia levels was 12 ppm when the animals were tested at five days of age. At 42 days of age, mean ammonia levels was 10 ppm.

Palpebral fissure length (PFL) was measured at five and then at 42 days of age using a stainless steel caliper with an LCD display and an accuracy of ± 0.02 mm (Neiko Tools, Klamath Falls, OR, USA).

2.3.2 Lacrimal production tests

Due to the small size of the eyes at 5 days of age, the regular STT1 was performed only at 42 days of age in another group of 10 chickens (20 eyes). Three alternative methods were used to test lacrimal production in a group of 30 chicks (60 eyes) with 10 chicks for each of the three different alternative tests at five and 42 days of age. Each test was performed by the same investigator (FMF) on both eyes: *oculus dexter* (OD or right eye); OS – *oculus sinister* (OS or left eye). All tests were noninvasive and caused minimal distress to the chicks. Time was monitored via a stop watch. STT-1 was performed by inserting a regular 35 mm-length/five mm-wide absorbent paper strip (DrogaVet, Curitiba-PR, Brazil) into the lower conjunctival

fornix (Figure 1) for one minute. The amount of wetting of the paper strips was measured as millimeters per minute. The mSTT1 was performed and prepared according to literature (Korbel and Leitenstorfer, 1998; Silva et al., 2013; Lima et al. 2015). In brief, a regular STT strip (DrogaVet, Curitiba-PR, Brazil) was aseptically transected with a scalpel blade and ruler (while still in the sterile package) into two 35 mm-long/2.5 mm-wide strips. The length of the wetting area was recorded in millimeters 1 min after it was placed inside the lower fornix (Figure 2 A and D). The phenol red cotton thread tear test (PRCTTT) was a commercially available kit (Zone-Quick; Menicon Inc., Clovis, CA, USA) with a 75-mm-long phenol-red impregnated thread with 3-mm bent end that was placed in the lower fornix (Figure 2 B and E) for 15 seconds (Saleh et al., 2006; Vashisht and Singh, 2011). When the phenol red (pH indicator) comes in contact with the slightly alkaline tears, it changes color from white to yellow-orange, yellow and then to red. The thread was removed after 15 seconds and the red portion was measured from the very tip regardless of the fold. The endodontic absorbent paper points tear test (EAPPTT) was performed according to the literature (Lange et al., 2012; Lange et al., 2014; Lima et al. 2015). The test consists of a small absorbent paper tip (Roeko Color, number 30, Langenau, Germany) which is used with endodontic treatment in dentistry and adapted to be used as a tear production test in small species of animals. It is read after one minute inside the lower fornix (Figure 2 C and F).



Figure 1. A representative 42-day-old broiler chick with a Schirmer Tear Test 1 (STT-1) strip inserted into the lower conjunctival fornix.

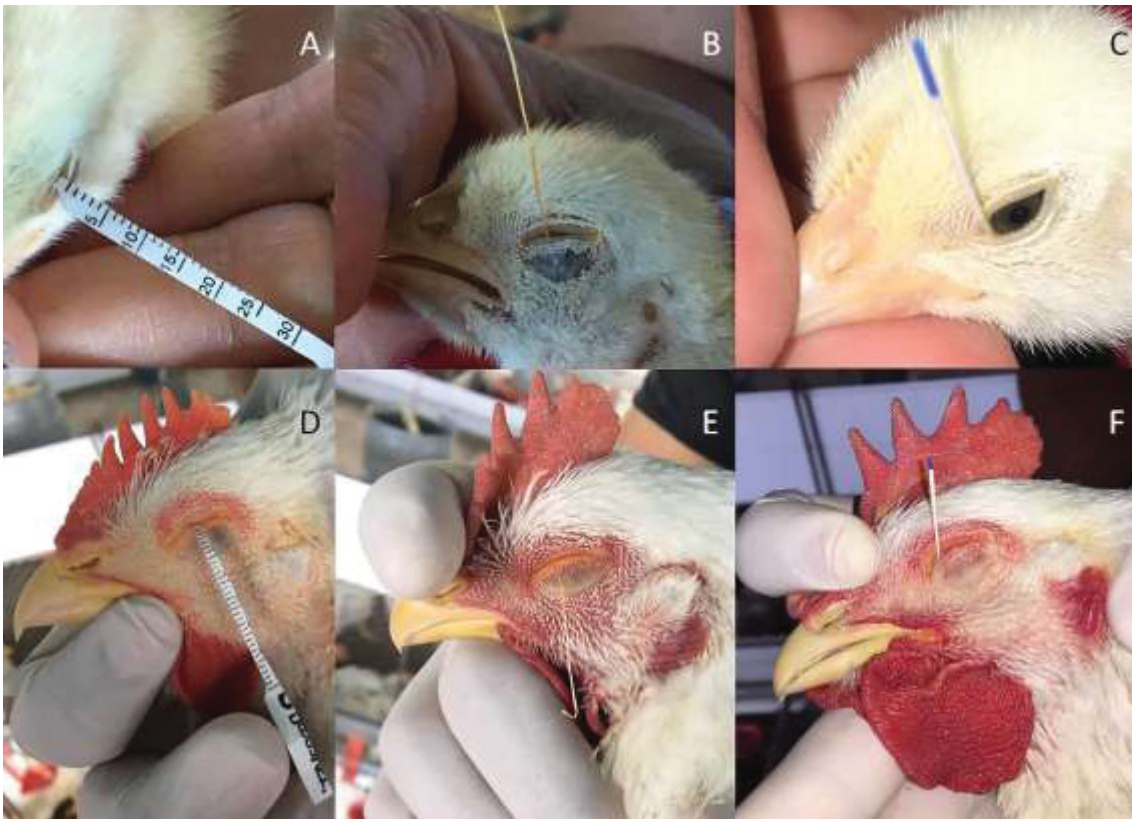


Figure 2. The use of the different alternative tear tests in representative five and 42-day-old broiler chicks, respectively. A and B: Modified Schirmer tear test 1 (mSTT1). C and D: Phenol red cotton thread tear test (PRCTTT). E and F: Endodontic absorbent paper points tear test (EAPPTT).

2.3.3 Statistical Analyses

Data obtained in this investigation were analyzed using descriptive and inferential statistics. A 2x2x3 factorial analysis was used to capture the effect age (2 levels: 5 and 42 days), eye side (2 levels: OD and OS), and tear test type (3 levels: mSTT1, PRCTTT, EAPPTT) and their interactions. A 2x2 factorial analysis was applied to PFL data to capture the effects of eye side, OD versus OS and age factor (5 days versus 42 days of age). Tukey's HSD All-Pairwise Comparisons Test was applied for the means with significant differences. *P*-values <0.05 were significant. Statistix®7 9.0 (Statistix 9.0 Analytical Software, Tallahassee, Florida, USA) software was used.

2.4 RESULTS

Mean STT1 for 42-day-old chicks was 11.4 ± 2.60 mm. More detailed descriptive data obtained with a regular STT1 were presented in Table 1; this test was performed in the additional group of 42-day-old-chicks only. Mean mSTT1 (SD) results were 5.07 ± 1.83 mm and 10.45 ± 2.58 mm for five and 42 day old chicks, respectively. Mean PRCTTT results were 12.37 ± 1.80 mm and 25.58 ± 4.8 mm, for five and 42 day old chicks, respectively. Mean EAPPTT results 7.03 ± 0.72 mm and 12.0 ± 0.92 mm, for five and 42 day old chicks, respectively. Descriptive and inferential statistics of all lacrimal tests is presented in Table 2. Lacrimal test type and age were the significant main effects ($p < 0.001$). Eye side was not a significant main effect ($p = 0.87$). The interaction between lacrimal test type and age also was significant ($p < 0.001$).

Descriptive and inferential statistics of PFL data is presented in Table 3. PFL significantly increases ($P < 0.001$) as the chicks grow older. Age was a significant factor for PFL ($P < 0.001$) but not eye side ($P = 0.404$).

Overall, results obtained with the different techniques showed that lacrimal production and PFL significantly increased between 5 and 42 days of age.

Table 1. Descriptive statistics of regular Schirmer Tear Test 1 (STT1) measurements in 42 day-old broilers

SCHIRMER TEAR TEST 1		
Results (mm)	Eye side	
	OD	OS
Mean	11.01	11.80
Std. Dev.	2.906	2.348
Std. Error	0.919	0.742
Minimum	7	8
Maximum	14	15
Variance	8.444	5.511
Coef. Var.	0.264	0.199

Table 2- Descriptive and inferential statistics for lacrimal production tests (SST1, mSTT1, PRCTT1, PRCTTT and EAPPTT) for 5 and 42 day-old broilers.

Test type	LACRIMAL PRODUCTION TESTS												Probability values	
	mSTT1		PRCTTT		EAPPTT		mSTT1		PRCTTT		EAPPTT			
Age	5 days of age						42 days of age							
Eye Side	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
Mean	4.25 ^D	5.75 ^D	12.30 ^B	12.44 ^B	7.33 ^{CD}	6.94 ^{CD}	9.90 ^{BC}	11.01 ^{BC}	26.67 ^A	24.50 ^A	12.02 ^B	12.04 ^B	0.943	0.943
Std. Dev.	1.814	1.603	1.636	2.068	0.577	0.776	1.853	3.162	5.68	3.937	0.943	0.943	0.298	0.298
Std. Error	0.574	0.463	0.517	0.689	0.333	0.274	0.586	1	2.319	1.607	0.298	0.298	0.079	0.079
Minimum	2	4	10	10	7	6	8	8	19	18	11	11	11	11
Maximum	8	9	15	16	8	8	13	18	33	28	14	14	14	14
Variance	3.292	2.568	2.678	4.278	0.333	0.603	3.433	10	32.267	15.5	0.889	0.889	0.889	0.889
Coef. Var.	0.427	0.279	0.133	0.166	0.079	0.112	0.187	0.287	0.213	0.161	0.079	0.079	0.079	0.079

P-values for the interactions: Eye side*Test type = 0.306; Eye side*Age = 0.560; Lacrimal test*Age = 0.001; Eye side* Lacrimal test*Age = 0.230.

Abbreviations: OD – *oculus dexter*; OS – *oculus sinister*; STT1 - Schirmer tear test 1; mSTT1 - modified Schirmer tear test 1; PRCTTT - phenol red cotton thread tear test; EAPPTT - endodontic absorbent paper points tear test; Std. Dev – Standard Deviation; Std. Error – Standard Error; Coef. Var. – Coefficient of Variance. Means within a row with different superscripts are different at $p < 0.05$.

Table 3. Descriptive and inferential statistics for palpebral fissure length (PFL) in 5 and 42 day-old broilers

Age	PALPEBRAL FISSURE LENGTH				Probability values
	5 days of age		- 42 days of age		
Eye side	OD	OS	OD	OS	0.404
Mean	4,264 ^A	4.1 ^A	9.173 ^B	9.124 ^B	0.001
Std. Dev.	0.216	0.214	0.571	0.537	
Std. Error	0.065	0.065	0.172	0.162	
Minimum	4	3.8	8	8	
Maximum	4.7	4.6	10	10	
Variance	0.047	0.046	0.326	0.289	
Coef. Var.					
Var.	0.051	0.052	0.062	0.059	

P-value for interaction: Eye side*Age = 0.656; **Abbreviations:** OD – *oculus dexter*; OS – *oculus sinister*; Std. Dev – Standard Deviation; Std. Error – Standard Error; Coef. Var. – Coefficient of Variance.

Means within a row with different superscripts are different at $p < 0.05$.

2.5 DISCUSSION

The absence of normal values for tear production in chicks in the scientific literature is likely related to the difficulty to perform tear production tests due to their small-sized palpebral fissure. Our results showed that their PFL is indeed too small to fit a regular-sized 0.5 mm wide STT strip. However, at 42 days of age their PFL is large enough to fit a regular STT. The fact that no parameters are available in the literature for adult chickens, however, is intriguing since the use of regular-sized STT strip is feasible (Figure 1). Several studies about lacrimal tests and its aqueous production have been conducted in human and veterinary medicine in healthy individuals (Messner, 2015; Lange et al., 2012; Lange et al., 2014; Lima et al. 2015; Saleh et al., 2006) and in eyes presenting different diseases (Benitez-del-Castillo et al, 2011). Previous studies have demonstrated that many diseases or management errors can increase or decrease

lacrimal production in poultry (Calnek, 2001; Miles et al., 2006; Freitas Neto et al., 2007; Zande and Kuhn, 2007; Arné et al., 2011; Maliselo and Nkonde, 2015). Avitaminosis A may be caused by inappropriate formulation of commercial diets or oxidation of vitamin resulting in glandular atrophy and decreased lacrimal production although its exact mechanism is unknown (Shane, 2005). Diagnosis of avitaminosis A can be difficult as it involves tissue collection and microscopic analysis (Shane, 2005). However, the use of lacrimal tests could help in the differential diagnosis of avitaminosis A. Husbandry issues like excessive ammonia concentration due to poor ventilation cause increase of lacrimal production by conjunctivitis and keratitis (Maliselo and Nkonde, 2015) and increase the susceptibility to respiratory diseases (Kristensen and Wathes, 2000). Our investigation was performed in an environment with low ammonia levels. It has been reported that chickens exposed to low levels of ammonia (25 ppm) may already show ocular changes. At 50-75ppm levels the signs may be exacerbated with presentation of ocular diseases such as corneal ulcers and abnormalities in the anterior chamber (Miles et al., 2006).

Although a lacrimal test can not directly diagnose infectious disease, it can be a complementary instrument to help the early detection. Examples of diseases that can cause conjunctivitis and excessive tearing are Newcastle and Marek's diseases, reoviruses, salmonellosis, aspergillosis, infectious coryza and infectious laryngotracheitis (Calnek, 2001; Freitas Neto et al., 2007; Zande and Kuhn, 2007; Arné et al., 2011; Shane, 2015).

This study established normal lacrimal test values for the aqueous portion of the tear film in younger and older broilers using different methods. Mean results obtained with PRCTTT in chicks were similar to those of adult Amazon parrots (12.5 ± 5.0 mm) (Storey et al., 2009). The results for the 42-day-old broiler were similar to larger Psittaciformes including macaws and cockatoos (25.4 ± 3.3 mm to 25.5 ± 5.2 mm) (Holt et al., 2006).

The mean mSTT1 values observed in the five-day-old chicks were similar to that of Psittaciformes (3.2 ± 2.7 to 7.5 ± 2.6 mm/min) (Korbel and Leitenstorfer, 1998), Screech owls (2 to 6 mm/min) (Harris et al., 2008), and Falconiformes (4.1 ± 2.7 to 14.4 ± 7.2 mm/min) (Korbel and Leitenstorfer, 1998). The mean STT1 values for the 42-day old chicks were somewhat similar to European birds of prey (12.47 ± 2.66 mm/min) (Barsotti et al., 2013), vultures (11.9 ± 3.3 mm/min) (Komnenou et al., 2013), ostrich (16.3 ± 2.5 mm/min) (Ghaffari et al., 2012), and bald eagles (14 ± 2 mm/min) (Kuhn et al., 2013). It was higher, however, than in large Psittaciformes including African gray parrots (8 ± 1.5 mm) and almost two times higher than values obtained in ducks (6.2 ± 2.2 mm) and geese (5.5 ± 2.6 mm) (Mood et al., 2017), Humboldt penguins (6.45 ± 2.9 mm/min) (Swinger et al., 2009) and small species such as lorries and conures (4.5 ± 1 mm) (Williams, 1994).

The mean EAPPTT value observed in the five-day-old chicks was similar to that observed in saffron finches (5.10 ± 0.26 mm) (Lange et al., 2014). As observed by our group (Lange et al., 2012) and other authors (Rajaei et al., 2014), EAPPTT tips are firmer and easier to handle than PRCTTT threads or mSTT or STT strips to the point that even one person alone is able to restrain the patient with ease, insert the paper into the conjunctival fornix without touching other structures, and read the test result. As shown on Table 2, the lacrimal test results with the smallest statistical variation were the EAPPTT, but this does not make the other tests unsuitable. Results obtained with mSTT presented the largest variation. This may be related to human error during regular STT strip sectioning resulting in different widths of the mSTT although care was taken to section the STT strips accurately. Industrialized manufacture of standardized mSTTs strips would help decrease potential variability in the acquisition process of the mSTT in the future. However, the regular STT1 in older chicks also showed a larger variance than EAPPTT.

Results obtained with each different technique and at each different age were significantly different to each other in most cases. Thus, these methods are not equivalent and reference values should be established for each method.

As seen in statistical analysis, there was a significant increase in tear secretion between 5-day-old and 42-day-old chicken. This can be explained by different stages of maturation of lacrimal gland during chick development. The same phenomenon has already been reported for humans and animals. In 1955, Sjogren demonstrated that tear secretion in humans increase with maturity. In dogs, researchers showed that tear secretion increases as neonates grow and reaches adult values when dogs are still in the end of the juvenile stage (Broadwater et al., 2010; Silva et al., 2013). The last age chosen to have tear production evaluated was the 42nd day as after this age the animals would be slaughtered for meat. Further studies may be conducted to examine chickens at other ages as well as different breeds of chickens.

In conclusion, tear secretion increases with age in broilers. Normal STT1 values for chickens reported here may serve as a parameter for future investigations. The three alternative tests for smaller eyes presented here are viable methods for measurement of tear production in chicks and their results may also serve as normal parameters for detecting eventual tear production abnormalities. Future investigations should address changes related to certain diseases or poor management conditions. Results obtained with EAPPTT showed the lowest coefficient of variation. EAPPTT was also easier to perform and animals showed less signs of discomfort during the whole procedure. Thus, we believe EAPPTT could be introduced on a routine basis for measurement of tear production in broilers.

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3. WINDOW COLLISIONS BY BIRDS IN THE SOUTH OF BRAZIL: EPIDEMIOLOGIC FACTORS, RADIOGRAPHIC AND NECROSCOPY FINDINGS.

3.1 ABSTRACT

Birds are among the most visually adept group of animals on the planet, however their inability to visualize and discriminate translucent glass structures results in hundreds of millions of deaths worldwide due to high-speed collisions. Despite reports of avian glass collisions in North America, few studies have been developed in order to better understand this scenario in South America nor evaluate radiographic and necropsy findings of affected birds. Data from 100 cadavers were examined radiographically and by necropsy, while 186 collision reports were analyzed for seasonality. A total of 34 different species of birds within 22 families were reported, with *Turdus rufiventris* (12), *Zenaida auriculata* (12) and *Columbina talpacoti* (10) being the most common species. Only seven (20%) migratory species were reported: *Chaetura meridionalis*, *Elaenia parvirostris*, *Florisuga fusca*, *Pitangus sulphuratus*, *Sporophila caerulescens* and *Turdus amaurochalinus*. Males (51) were more frequently reported than females (5), and 50.1% of the males had active gonads on necropsy. The most common radiographic lesion, noted in 19.5% of the animals, was loss of coelomic definition, suggestive of hemorrhage. Prevalent necropsy findings included skull hemorrhages (77.3%) and encephalic contusions (64.4%) followed by coelomic hemorrhages (40.7%). Most collisions (61, 32.8%) occurred during spring, the most common breeding season of avian species in Brazil. Cranioencephalic trauma was identified as the most reported cause of mortality in glass collision accidents. Migration does not seem to be the main predisposing factor for window collisions by birds in South America. Increased activity and aggression related to breeding season, especially in males, may be a more important predisposing factor for window collisions accidents.

KEY-WORDS: Ornithology. Glass. South America. Cranioencephalic trauma. Reproductive season.

3.2 INTRODUCTION

The visual system of most avian species is highly-developed (Moore et al., 2013; Tyrrell et al., 2013, Baumhardt et al., 2014, Moore et al., 2019). As a result, behavioral ecology is vision dominated, with most species depending on high visual acuity and wide fields of view for successful foraging, anti-predator, and communication with conspecifics (e.g. posture, displays, movements for courtship or vigilance signaling) (Osorio and Vorobyev, 2008; Moore et al., 2015; Moore et al., 2017a; Moore et al., 2017b; Bertin et al., 2018; Tyrrell et al., 2019). Color vision among birds is in many ways superior to that of mammals, including humans, as tetrachromatic and even pentachromatic spectra have been documented (Moore et al., 2012; Tyrrell et al., 2019). The visual spectrum of some species is more comprehensive than that of the human eye, enabling visualization of wavelengths not visually apparent to humans, such as those within the ultraviolet spectrum.

Despite having such advanced visual systems, collisions against anthropogenic structures (e.g. commercial and residential buildings) is among the most frequent causes of free-living bird mortality, second only to cat predation (Loss et al., 2012a; Loss et al., 2012b; Klem, 2015; Santos et al., 2017). A major reason for such frequent collisions is the increasingly popular use of translucent glass for windows, which are not only aesthetically pleasing but also contributes to the health and well-being of humans (Bonmati-Carrion et al., 2014). Glass-related collisions are estimated to be responsible annually for 365 to 988 million bird deaths in the United States alone (Loss et al. 2014), and 16 to 42 million in Canada (Machtans et al., 2013). Similar reports exist from Costa Rica (Menacho-Odio, 2015), Mexico (Cupul-Magaña, 2013), Colombia (Agudelo-Álvarez, 2010) and Argentina (Rebolo-Ifrán, 2019). The reported mortality rates likely underestimate the number of collisions due to the increased risk of

predation of concussed or unconscious birds, carcass deterioration, and consumption by scavengers (Klem et al., 1990; Klem et al., 2004; Klem et al., 2009; Hager et al., 2012; Parkins et al., 2015).

However, there are scattered studies regarding the occurrence of window collisions by birds in South America. In Brazil, bird collision has been evaluated only in the Southeast Region, in a natural reserve in the state of Minas Gerais (Santos et al., 2017) and at a university campus in Sao Paulo (Brisque et al., 2017). The aim of the present study is to evaluate the occurrence of window collisions by birds and the impact on their health at the Federal University of Parana (UFPR) in Curitiba, Brazil. Additionally, we introduce a website designed for collecting data from public reports of bird collisions and present an initial analysis of the submitted data.

3.3 MATERIALS AND METHODS

Birds that were presented to the Veterinary Teaching Hospital (VTH) of Federal University of Parana, Curitiba, Brazil with a clinical history of known trauma due to glass collision on buildings were included in this study. Both live and dead animals were accepted from local citizens as a part of an outreach initiative. Birds that collided with glass but were still alive were received by the VTH's Zoological Medicine service and treated according to internal protocol. Bird cadavers received and birds that died during treatment were radiographed and necropsied at the same institution.

Additional information from other bird collisions were also collected indirectly via a website that was developed by the Federal University of Paraná for general Brazilian citizens to report bird glass collisions (<http://www.prppg.ufpr.br/entreavidaeovidro/>), which also was a part of an outreach initiative promoted by the Federal University of Paraná.

3.3.1 Radiographs

Whole body radiographs of bird cadavers were obtained by using two projections, right lateral and ventrodorsal. The ventrodorsal projection was repeated three times, including different cranium positions: rostro caudal, extended, and flexed. Radiographs were performed using a DIAFIX 500mA/125kV digital radiograph machine set at approximately 44kV, 100mAs, and 0.04s for an average 100-gram bird. These values were slightly adapted according to different bird sizes and weights for a better image. A boarded veterinary radiologist (Brazilian College of Veterinary Radiology and Diagnostic Imaging) trained in exotic radiology, evaluated the radiographs in this study.

3.3.2 Necropsies

Necropsies were performed using routine protocols for birds (Rae, 2003). In brief, birds were placed in dorsal recumbency, keel and pectoral muscles were removed cranially after resecting the ribs and the shoulder girdle. The contents of the coelomic cavity were evaluated for the presence of free fluid and hemorrhage. Followed by inspection of the liver and heart. Esophagus and trachea were removed from the cranial extremity extending to the crop. Gonads and kidneys were examined within the coelomic cavity. In addition, gonads were identified and visually ranked as active or inactive, testicles were considered active if they were enlarged and ovaries were considered active if they had visible follicular hierarchy (presence of pre-vitelogenic and vitelogenic follicles). Afterwards, the heart, gastrointestinal tract, lungs and trachea were evaluated out of the carcass. The head was carefully examined, prior to removing the skin from the skull. The dorsal calvarium was then carefully removed with scissors and the brain evaluated within the skull. The pneumatic skull bones, sinuses and encephalon were examined for signs of hemorrhage.

3.3.3 Website

The website specially designed for this investigation provided educational information to the public regarding bird window collisions. The website also has guidelines about the inclusion criteria, a submission form to report collisions in detail. The submission form included name, e-mail address of the reporting individual, location and date of the collision, bird species, type of window and an optional space to upload a picture (i.e. dead bird, feather dust imprint left on the window after the accident). Printed forms were also available physically in the VTH for manually report glass collisions even if the reporting individual did not bring the affected animal.

3.3.4 Statistical analyses

After the data was collected, the necropsy records and radiographs findings were prepared for statistical analysis. The absolute frequency of species, families and migratory condition of birds that suffered window accidents were calculated. Data of collisions were grouped according to the season of year they occurred and tested for normality through Shapiro-Wilk test. Since the resulting p-value of the Shapiro-Wilk Test was below 0.05, the data significantly deviated from a normal distribution. Thus, the data were compared through Kruskal-Wallis analysis. Potential differences in proportion of accidents in dry and rainy seasons were compared with a Pearson's chi-squared test.

3.4 RESULTS

3.4.1 Number of animals investigated

A total of 106 collisions were reported through the website. Additionally, 39 manually filled reports were received. Therefore, a total of 145 reports were associated with the 100 cadavers received in the UFPR's VTH. Duplicities were excluded: 4 cadavers that were reported in the website but also delivered to VTH-UFPR for the investigation. Specimens

without an exact date for the accident (59 cadavers) were excluded for seasonal studies. A total of 186 collisions with exact dates provided were available to be analyzed.

3.4.2 Taxonomic identification

The 100 birds that were received at UFPR's VTH between September 2017 and August 2019 consisted of 34 different species within 22 families. A high degree of autolysis was present in 6 cadavers, and thus taxonomic identification was not possible. According to the classification of Somenzari et al. (2018), all birds were native or common non-native species to Brazil, with 6 out of 32 species (18.8%) considered migratory. The bird species involved in glass collisions along with their respective migratory habits are summarized in Table 01. The most prevalent orders were Columbiformes and Passeriformes, the most affected families were Thraupidae and Columbidae, with 5 and 4 species and a total of 17 and 25 birds, respectively. The most common species were *Turdus rufiventris* (12), *Zenaida auriculata* (12), *Columbina talpacoti* (10) (Figure 1 A-C), *Elaenia parvirostris* (8), and *Tangara sayacu* (7); 22 species had only a single bird presented. Males (51) outnumbered females (5), and the sex was unable to be determined in 44 birds due to autolysis of the gonads and/or absence of gross sexual dimorphism. Half of the males had active gonads in necropsy (50.1%), noted by the increased of the size of the testicles, while no females had active ovaries. Cadavers received with accurate annotation of the day of the collision were used for seasonality analysis in association with the website reports. All the radiographed birds were necropsied, but not all necropsied birds were radiographed.



Figure 1 - Most prevalent species affected by glass collision - (A) *Turdus rufiventris*, (B) *Zenaida auriculata*, and (C) *Columbina talpacoti*. Courtesy of Zig Koch.

Table 1. Absolute frequency of species, families and migratory condition of birds that suffered glass collisions

Species (Scientific name)	Common name	n	Migration	Family	n
<i>Turdus rufiventris</i>	Rufous-bellied thrush	12	NM	Turdidae	3
<i>Zenaida auriculata</i>	Eared dove	12	ND	Columbidae	4
<i>Columbina talpacoti</i>	Ruddy ground dove	10	NM	Columbidae	4
<i>Elaenia parvirostris</i>	Small-billed elaenia	8	PM	Tyrannidae	2
<i>Tangara sayaca</i>	Sayaca tanager	7	NM	Thraupidae	5
Autolysis (6)	NA	6	NA	NA	NA
<i>Brotogeris tirica</i>	Plain parakeet	6	NM	Psittacidae	1
<i>Sicalis flaveola</i>	Saffron finch	6	NM	Thraupidae	5
<i>Zonotrichia capensis</i>	Rufous-collared sparrow	3	NM	Passerellidae	1
<i>Cacicus haemorrhous</i>	Red-rumped cacique	2	NM	Icteridae	2
<i>Furnarius rufus</i>	Rufous hornero	2	NM	Furnariidae	1
<i>Patagioenas picazuro</i>	Picazuro pigeon	2	NM	Columbidae	4
<i>Tangara palmarum</i>	Palm tanager	2	NM	Thraupidae	5
<i>Baryphthengus ruficapillus</i>	Rufous-capped motmot	1	NM	Momotidae	1
<i>Chaetura meridionalis</i>	Sick's swift	1	M*	Apodidae	1
<i>Chiroxiphia caudate</i>	Blue manakin	1	NM	Ilicurinae	1
<i>Chloroceryle americana</i>	Green kingfisher	1	NM	Alcedinidae	1
<i>Colaptes campestris</i>	Campo flicker	1	NM	Picidae	2
<i>Columba livia</i>	Rock dove	1	NM	Columbidae	4
<i>Cyclarhis gujanensis</i>	Rufous-browed peppershrike	1	NM	Vireonidae	1
<i>Florisuga fusca</i>	Black Jacobin	1	PM*	Trochilidae	1
<i>Molothrus bonariensis</i>	Shiny cowbird	1	NM	Icteridae	2
<i>Passer domesticus</i>	House sparrow	1	NM	Passeridae	1
<i>Piaya cayana</i>	Squirrel cuckoo	1	NM	Cuculidae	1
<i>Picumnus nebulosus</i>	Mottled piculet	1	NM	Picidae	2
<i>Pitangus sulphuratus</i>	Great kiskadee	1	PM*	Tyrannidae	2
<i>Pygochelidon cyanoleuca</i>	Blue-and-white swallow	1	NM	Hirundinidae	1
<i>Pyrrhocomma ruficeps</i>	Chestnut-headed tanager	1	NM	Thraupidae	5
<i>Ramphastos dicolorus</i>	Green-billed toucan	1	NM	Ramphastidae	1
<i>Rupornis magnirostris</i>	Roadside hawk	1	NM	Accipitridae	1
<i>Setophaga pitiaiyumi</i>	Tropical parula	1	NM	Parulidae	1
<i>Spinus magellanicus</i>	Hooded siskin	1	NM	Fringillidae	1
<i>Sporophila caeruleascens</i>	Double-collared seedeater	1	PM*	Thraupidae	5
<i>Turdus albicollis</i>	White-necked thrush	1	NM	Turdidae	3
<i>Turdus amaurochalinus</i>	Creamy-bellied thrush	1	MPR	Turdidae	3
34 species		100 birds		22 families	

Number of birds (n). Non-migratory (NM), migratory (M), partially migratory (PM) and not defined (ND). * Species suggested to be further studied (Somenzari et al. 2017).

3.4.3 Necropsy findings and radiographic lesions observed

The lesions observed during necropsies and radiographic evaluations are summarized in Table 02.

Table 2. Necropsy and radiographic findings

	<i>Necropsies</i>				<i>Radiographs</i>			
	Males n=51	Female s n=5	NI n=44	Total n=100	Males n=44	Female s n=4	NI n=36	Total n=82
<i>Crop rupture</i>	3 (3%)	1 (1%)	2 (2%)	6 (6%)	NA	NA	NA	NA
<i>Coelomic hemorrhage</i>	19 (19%)	4 (4%)	10 (10%)	33 (33%) 33/81 (40.7%)*	NA	NA	NA	NA
<i>Encephalic contusion</i>	29 (29%)	4 (4%)	14 (14%)	47 (47%) 47/73 (64.4%)*	NA	NA	NA	NA
<i>Skull hemorrhage</i>	37 (37%)	4 (4%)	17 (17%)	58 (58%) 58/75 (77.3%)*	NA	NA	NA	NA
<i>Radiographic lesion</i>	NA	NA	NA	NA	19 (23.1%)	2 (2.4%)	20 (24.4%)	41 (50%)
<i>Beak fracture</i>	5 (5%)	0 (0%)	3 (3%)	8 (8%)	3 (3.6%)	0 (0%)	4 (4.8%)	7 (8.5%)
<i>Fracture Appendicular skeleton</i>	1 (1%)	0 (0%)	1 (1%)	2 (2%)	4 (4.8%)	1 (1.2%)	3 (3.6%)	8 (9.7%)
<i>Fracture Axial skeleton</i>	NA	NA	NA	NA	0 (0%)	0 (0%)	4 (4.8%)	4 (4.8%)
<i>Cranial fracture</i>	NA	NA	NA	NA	3 (3.6%)	0 (0%)	3 (3.6%)	6 (7.2%)
<i>Luxation</i>	NA	NA	NA	NA	3 (3.6%)	0 (0%)	0 (0%)	3 (3.6%)
<i>Loss of coelomic definition</i>	NA	NA	NA	NA	9 (10.9%)	1 (1.2%)	6 (7.2%)	16 (19.5%)

*- Number corrected due to autolysis

3.4.4 Radiographs

Eighty-two birds were evaluated radiographically, forty-one (50%) of the animals were diagnosed with a lesion radiographically consistent with trauma. Sixteen birds (19.5%) had a loss of coelomic definition suggestive of hemorrhage (Figure 2A). Cranial fractures were noticed in six cases (7.2%) (Figure 3A), and four cases (4.9%) had extra-cranial fractures in the axial skeleton. Eight fractures in appendicular skeleton (9.7%) were noted (Figure 2B), with two of the birds having multiple fractures. Seven beak fractures (8.5%) were also noted (Figures 3B and 4A).

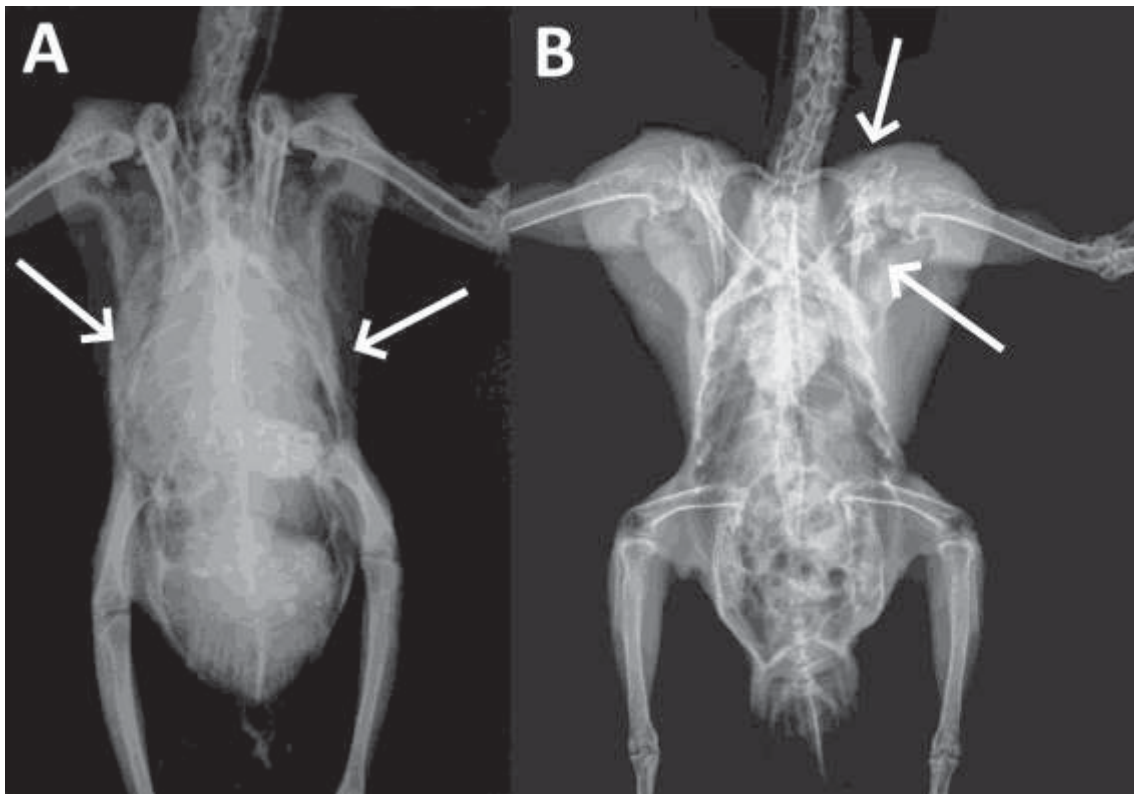


Figure 2 – Representative radiographic findings. (A) Ventrodorsal view showing loss of definition in the thoracic cavity of a *Tangara palmarum*, suggestive of thoracic hemorrhage. Coelomic viscera caudal to the liver can still be delineated, and the presence of grit in the ventriculus and abdominal air sacs can be visualized. (B) Ventrodorsal radiograph of a *Zenaida auriculata* showing an appendicular skeleton fracture affecting left scapular girdle – scapula and coracoid.

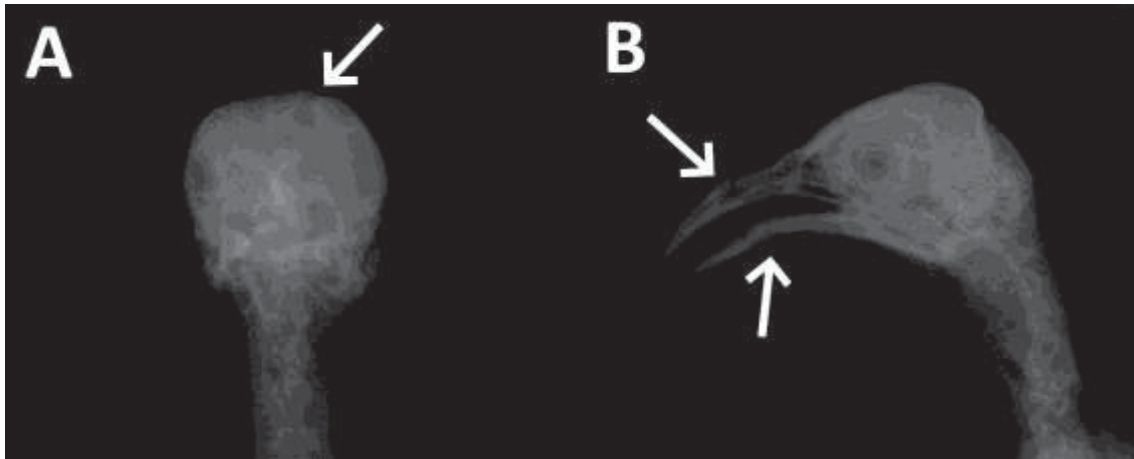


Figure 3 - Representative radiographic findings. (A) Cranioventral view of a cranial fracture in a *Zootrichia capensis*. (B) Lateral radiograph depicting a beak fracture affecting both gnathoteca and rhinoteca of a *Zootrichia capensis*.

3.4.5 Necropsy

The most frequent lesions observed on necropsy were skull hemorrhages (58%) and encephalic contusions (47%) among all necropsies realized, including contusive and concussive lesions. Correcting these values considering the animals with moderate to high degree of autolysis, in which necroscopic observations were compromised, skull hemorrhages (intraosseous) (Figure 4B) were present in 58/75 (77.3%) of the skulls and encephalic hemorrhage (brain contusion) in 47/73 (64.4%) of them. In several cases, the contusions were present in the cranial and caudal aspects of the brain (Figure 4C and 4D). Coelomic hemorrhages were also prevalent, noticed in 33% of the birds – corrected to 33/81 (40.7%) (Figure 4E). Fractures and ruptured organs were less common; beak fractures were noted in eight cases, limb fractures in two cases, and crop rupture in six animals (Figure 4F).

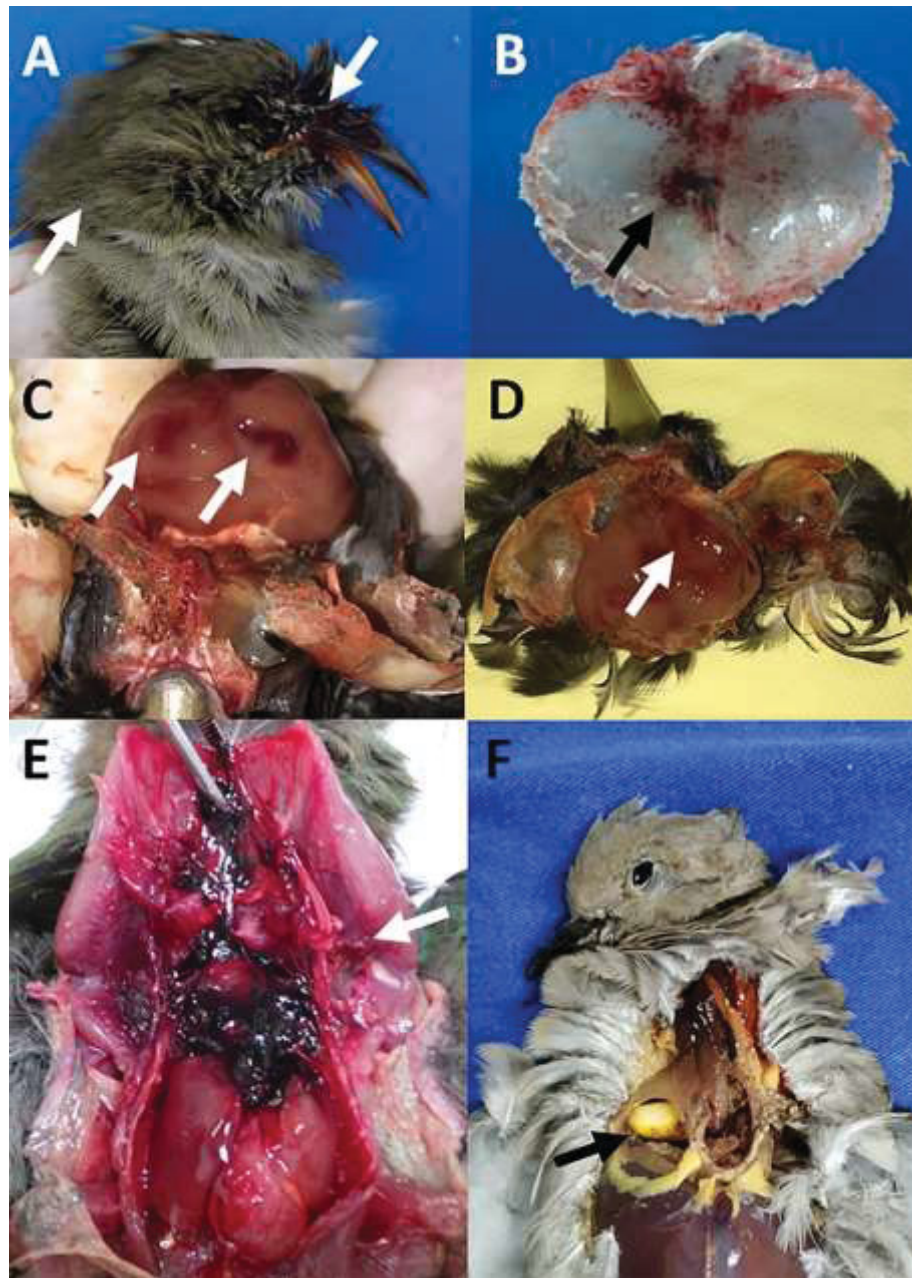


Figure 4 - Frequent lesions found during necropsy. (A) Beak fracture (arrow) affecting both rhinotheca and gnathotheca in an *Elaenia parvirostris*. (B) Skull (intraosseous) hemorrhage (arrows) in a *Rupornis magnirostris*. (C) Encephalic hematoma in a rostral location (arrows) in a *Cacicus haemorrhous*, and (D) caudal location (arrow) in the same bird. This pattern is typical of a coup-counter coup injury. (E) Moderate hemorrhage affecting the right and left pleural cavities in a *Zenaida auriculata*. No pulmonary hemorrhage, liver or heart lacerations were diagnosed in this animal. (F) Crop rupture of a filled crop in a *Zenaida auriculata*.

3.4.6 Website and Forms

Website reports were made from six different Brazilian states, all of them within the same time zone (UTC/GMT -3 hours). The additional data included information about the glass structures and attempts of species identification. These data were not included in our report due to their high level of inaccuracy. Most reports from the website (76/106, 71.7%) came from the metropolitan area of the city of Curitiba (including smaller cities). Date of the reports on the website were used to describe the seasonality of collisions, combined with 39 additional reports provided directly at the VTH through clients, staff or students that manually have filled printed forms. An average of 15.5 ± 6.3 collisions were reported monthly, with the most frequent month being November (29) and the least frequent being July (5). Most collisions occurred during spring totaling 61 (32.8%), followed by autumn, summer and winter with 23.7%, 24.2% and 19.3%, respectively (Figure 5).

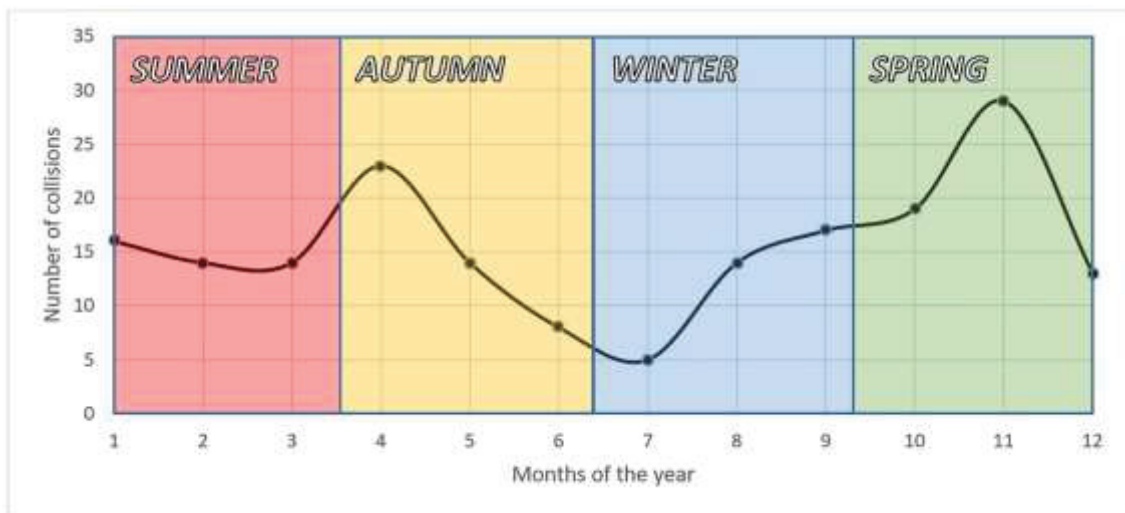


Figure 1 - Distribution of glass-collisions throughout the year. Most collisions occurred in November, the spring and rainy season in Brazil, while a reduction in collision numbers is evident in winter.

3.4.7 Influence of the season of year, dry *versus* rainy season

A significant difference ($p < 0.0001$) existed between spring and all the other seasons. However, there was no significant difference between rainy and dry seasons ($p = 0.1$).

3.5 DISCUSSION

Collisions with windows occur due to the inability of birds to detect glass in their flight path. The resultant impact is often high-speed, with the head receiving a large amount of the impact force. Some possible factors contributing to window collisions are visualization of a natural landscape through the glass, mirrored glass that reflects the landscape and is interpreted by the bird as continuity of the environment, or even a reflection of its own image interpreted as another bird initiating an aggressive encounter. Parkins et al. (2015) noticed that many buildings surrounded by landscapes including flowers and shrubs promoted reflection of this vegetation in glass windows, and that these buildings were associated with the highest number of collisions. Borden and Lockhart (2010) also noticed a greater risk of collision in windows associated with the presence of trees within 5 meters from the building. An increase in mortality is also noticed when feeders are placed near the glass (Kummer and Bayne, 2015; Kelm et al., 2004). However, fatal collisions occur worldwide in cities and rural areas, and all sizes and shapes of windows are at risk of collision for birds, regardless of the time of day, season, or weather conditions (Bayne et al., 2012; Loss et al., 20012a, Hager et al., 2013; Loss et al., 2014, Cusa et al. 2015). The exact time of the day in which an accident occurred was not recorded for most of the carcasses received. While nighttime lighting is frequently cited as a factor contributing to building collisions, few formal assessments have been conducted (Hager et al., 2008; Parkins et al., 2015).

Bird-related factors should also be considered, such as species, flight patterns, flocking patterns, visual capabilities, activity patterns and seasonality, among many others.

Columbiformes and Passeriformes are the most commonly reported orders, corroborating with the present study. More specifically, in Brazil, Santos et al. (2017) also reported Columbidae and Thraupidae as the most affected families within the Columbiformes and Passeriformes orders. Some pigeons have been shown to exhibit a keen kinesthetic sense of their body and wings in relation to visible complex obstacle-laden ecosystem (Ros et al., 2017). However, two members of the bird family Columbidae were among the top three bird species with the most window accidents recorded, Eared doves (*Zenaida auriculata*) and Ruddy ground doves (*Columbina talpacoti*), both among the most common dove species in the Neotropics. Some synanthropic species, such as the Rock Dove (*Columba livia*) and House sparrow (*Passer domestic*), are suspected to learn to avoid windows and are not frequently observed colliding worldwide (Klem, 2014). Additionally, flight behavior toward vegetation or feeders, including those of which are near glass surfaces where, like feeders close to windows, may be low velocity such that if they strike the window it is with sub-lethal force, enabling them to learn to avoid the space thereafter (Klem, 2014, Ros et al., 2017). This learned behavior may not occur in Eared doves and Ruddy ground doves. Alternatively, given the high correlation between the number of accidents and the population size in each location, the occurrence of Passeriformes and Columbiformes collisions may be solely a reflection of population density as are the most common orders found in areas where buildings are present (Loss. et al., 2014).

Santos et al. (2017) found that collisions are more common during rainy periods in Brazil, when the foraging and reproductive behaviors are increased and visibility is decreased. In the present study, even though collisions were numerically higher during the month of November, which is the middle of the local rainy season (Pereira et al., 2008), there was no statistical difference between rainy and dry seasons. Brisque et al., (2017) found no difference in collision rates between seasons in the state of Sao Paulo. Contrarily, we found that during the spring, accidents were reported at a significantly higher rate compared to all other seasons.

These findings agree with those in North American, where collisions tend to increase during the migration periods of spring and autumn (Gelb and Delacretaz, 2006; Gelb and Delacretaz, 2009; Borden and Lockhart, 2010; Parkins et al., 2015; Kahle et al., 2016). However, in this study, only a minority of the necropsied individuals that suffered collisions were migratory (13/100). In fact, only one migratory species (*Elaenia parvirostris*) was present among the five most common species reported here. In the present study, a large number of collisions occurred among juveniles and reproductively active birds, particularly males. Therefore, this temporal distribution of collisions may be more related to the reproductive season when males are searching and competing for mates and have increased aggression between individuals or to their own reflection in glass. However, many birds (44) could not be sexed due to general autolysis or the inability to identify the gonads on necropsy because of moderate-marked autolysis or, in a few cases, coelomic hemorrhage, which usually accumulated in the dorsal coelom around the gonads. If the majority of these unsexed birds were indeed adult males and even some juvenile males, the latter theory would still hold true. Nevertheless, it is important to consider that if a large portion of those birds were female, seasonal aggression for adult males or dispersal mobility juveniles may not be a major contributing factor for window collisions. Hager and Craig (2014) analyzed bird-window collisions during breeding seasons in North America and found accidents to be high in adults of the least abundant species and juveniles of the most abundant species. They discuss this pattern to be a result of breeding adults engaging in high velocity social interactions such as chasing, territory establishment, and defense, whereas post-fledging individuals are entering the population through dispersal mobility. As discussed by the authors, this explains the reduced number of collisions in winter and summer, when territory settlement is finished and reproduction behaviors are mostly brooding of eggs and nestlings, representing reduced mobility of the adult birds, also observed in this study.

The flying behavior of the species also plays a role in window-collision events, with “tunnel flyers” that fly between small spaces within dense environments being more prone to glass collision. Birds with elliptical wings, such as robins and sparrows, are also more affected due to their flying characteristics compared to high-speed, game bird and slotted high-lift wings. Additionally, birds living in mixed open and closed habitats (i.e. forest independent birds) are able to better identify obstacles in open areas such as buildings and glass, avoiding collisions with more success (Klem, 2015). In fact, Santos et al., (2017) concluded that forest dependent species have the greatest influence on reducing liability to glass collision.

It is estimated that approximately half of bird collisions with glass result in death (Klem, 1990; Veltri and Klem, 2005; Gelb and Delacretaz, 2009; Kummer and Bayne, 2015; Brisque et al., 2017) In this study, the potential mortality of this type of accident was close to 100%, as almost every bird received by the Zoological Medicine service did not survive despite clinical treatment. It is suspected that birds referred for veterinary care in Curitiba had been severely debilitated with an unfavorable prognostic. The gross necropsy findings in this study varied from no visible damage or simple excoriations to broken bones and superficial or deep hemorrhages. Although cervical lesions may be expected following trauma, no cervical fractures were found in the present study or by Veltri and Klem (2005).

Traumatic cranial injury was the most prevalent lesion found in necropsy, affecting more than 77% of the birds, followed by encephalic hemorrhage, affecting 64.4% of the birds. The fact that brain contusions were present in the cranial and caudal aspects of several brain tissues could indicate that a coup-countercoup type of contusion have occurred. In this type of injury, acceleration-deceleration results in the formation of lesions in the cranial (propelled forward at the point of impact) and caudal aspects of the brain (when it moves back during deceleration). This makes sense when considering window collisions. Veltri and Klem (2005) examined two brains histologically and found mainly hemorrhage in the cerebrum and

cerebellum. Our findings of 64.4% of birds having traumatic brain injury may have been much higher if histological examinations for microscopic hemorrhage were performed, especially considering that 77.3% of the bird had cranial injuries. Small lesions not visible on gross necropsy may still affect nerve fiber communication, breakage of blood vessels, rupture of the blood-brain barrier, subdural bleeding evolving to cranial edema and increase in intracranial pressure. Thus, necroscopic evidence of cranioencephalic trauma probably explains the neurological clinical signs (apathy, responsiveness decreased) described in other studies (Klem 1990; Veltri and Klem, 2005) and probably was the most common cause of death for the birds analyzed in the present investigation.

Radiographs were extremely sensitive for diagnosing skeletal injuries, such as limb, beak and cranium fractures. Loss of coelomic definition, suggestive of coelomic effusion or hemorrhage, was noticed in 19.5% of the birds, while 33% of them presented coelomic hemorrhage during necropsy. Coelomic hemorrhage is not a commonly reported injury in birds that suffered collisions with windows (Klem, 1990; Veltri and Klem, 2005). Increased coelomic radiopacity was usually not evenly distributed in affected birds. Coelomic organs may lose their silhouette due to fluid opacity, but in a few cases it was still possible to determine contours and contrast between air sacs and organs. In VD projection it was possible to determine the opacity location (thoracic and/or coelomic) and distribution between contralateral sides, while in lateral projection it was sometimes possible to suggest the presence of fluid ventrally or dorsally. In all 16 birds, fluid opacity was present in thoracic cavity, while in 13 birds coelomic cavity was also affected. In 12 of these 13 birds, it was possible to subjectively suggest fluid more concentrated in the thoracic cavity, while in one bird the cardiac silhouette was specifically increased. . These data corroborates with necropsy findings, where all birds with hemorrhage had blood present specially within the the right and left pleural cavities. Rapid cardiac compression, formerly named thoracic compression, is a standard technique of bird euthanasia

in field activities (Engilis et al. 2018), and is based on the application of digital pressure over the heart of the bird leading to rapid rupture of the thin-walled regions of the vena cava and/or right atrium (Paul-Murphy et al., 2017). This technique was demonstrated to result in coelomic, pericardial, or perihepatic hemorrhage associated with rapid cessation of pulse, breathing and cerebral activity (Paul-Murphy et al., 2017). Although the most common location of bleeding is between the heart and the cranial margin of the right liver lobe, diffuse celomic hemorrhage may occur (Paul-Murphy et al., 2017). These findings are consistent with the radiographic and necropsy findings in the birds in this study. The high-velocity impact of the bird with the glass structures may lead to rupture of heart or other circulatory tissue, especially in the thoracic cavity, resulting in thoracic hemorrhage. Depending on the vector force or velocity of the impact, the damage may be more extensive and diffuse celomic hemorrhage may be present. None of the necropsied birds presented with any gross damage to the lungs or liver, and diagnosis of a ruptured heart or vena cava in fresh or frozen cadavers may be difficult (Paul-Murphy et al., 2017). In animals where thoracic hemorrhage was diagnosed, death due to hypovolemic shock must be considered, as it would occur rapidly. Therefore, through radiography it is not possible to definitively diagnose a traumatic origin of the lesions, it is possible to affirm whether or not hemorrhage occurred in the thoracic and/or coelomic cavity. The significance of this hemorrhagic lesions should be further investigated in the future.

Latin America studies of bird collisions report more than a hundred species to have been victims of collisions with glass, with almost five fatal incidents per month, although most of these reports are limited to a specific area or a few buildings (Cupul-Magaña, 2003; Agudelo-Álvarez et al., 2010; Menacho-Odio, 2015; Ocampo-Peñuela et al., 2016; Brisque et al., 2017; Santos et al., 2017). Even if studies were expanded to include larger areas or more building, citizens generally underestimate the mortality of birds due to their lower efficiency to identify and collect animals compared to researchers (Bracey et al., 2016).

Study limitations include not having estimated the age in all individuals and a lack of histopathologic investigation of the lesions. However, the cadavers investigated were received with varying storage times. Several cadavers were kept refrigerated but numerous were not. This would have limited the quality of the evidence provided in such analyses. Time also may affect the quality of necropsies. For instance, the observable amount of blood and fluid in the mouth and nasal cavities may decrease due to drying (Veltri and Klem, 2005). In the future, an active search for recently deceased birds would make such an approach possible. Another limitation was the fact that in terms of epidemiologic studies the geographical location of the population of birds studied was not uniformly distributed. Nevertheless, bird species reported on the website were not considered in the investigation due to potential mistakes in the identification.

In the present study, many citizens did discuss with the researchers about the problems with bird collisions, but the frequency of bird presentation by the citizens did not substantially increase. Nonetheless, in the future by advertising nationally and providing a website for reporting bird collisions, more people will be able to be reached and more data will become available.

This is the first investigation of bird window collisions performed in the south of Brazil. Our study corroborates with other Latin American publications that bird collisions against glass windows is highly prevalent in rural and urban areas, although it is probably still underdiagnosed due to many factors (Cupul-Magaña, 2003; Agudelo-Álvarez et al., 2010; Menacho-Odio, 2015; Ocampo-Peñuela et al., 2016; Brisque et al., 2017; Santos et al., 2017; Rebolo-Ifrán et al., 2019). There are species more prone to collisions, but regional occurrence of species also plays a significant role and is difficult to understand without a much larger sample size. The high prevalence of collisions among breeding birds (25%, 51% of the males) may go beyond just an individual death, including also the likely death of their young that

depend on them. Thus, a single collision-related death of a breeding bird likely equates to the death of far more than just a single individual, suggesting an impact on avian populations greater than what historical reports have indicated. The most important lesion observed in radiographs and necropsies are cranial trauma promoting neurological disorders with an unfavorable prognosis. Histopathological examination of brain tissue would likely result in an even higher prevalence of cerebral and cerebellar hemorrhage, justifying the high mortality rate. Another possible cause of death that must be considered according to the results of this study is a rapid cardiac compression resulting in thoracic hemorrhage and hypovolemic shock, which may have occurred in at least 30% of the birds in this study. Environmental education of the general public is essential to help prevent window collisions, but also to identify, rescue and help affected birds. Larger future studies may be used to help determine the ecological and bird factors contributing to glass window collisions. Additionally, future studies evaluating the specific visual parameters related to visualization of windows may be able to help researchers understand avian visualization of windows and may be used in the development of vision-based preventative measures.

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5. ANEXOS – Information about the scientific papers already published and/or accepted for publication (as first author or as a co-author)

Schirmer's I, modified Schirmer's I, phenol red thread, and paper point tests: a comparative study for tear production measurement techniques in broiler chicks (*Gallus gallus domesticus*)

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ABSTRACT Problems with indoor husbandry and immunological challenges are some of the stressors that broilers may face which can affect production. Infectious diseases can compound the effects of excessive concentrations of ammonia causing respiratory and ocular inflammation and increased lacrimal production in broiler chickens. The objectives of this investigation were to establish normal values of tear production in chicks and compare the practicality of the different methods. Palpebral fissure length (PFL) and tear production were evaluated in Cobb 500 chicks at 5 d old and then at 5 d of age by the following methods: modified Schirmer's test I (mSTT1), phenol red cotton thread tear test (PRCTTT), and standardized endodontic absorbent paper point test (EAPPTT). Ten chicks at the age of 5 and 42 d were evaluated for each method. In addition, at 42 d, when the palpebral fissure permitted, a regular-sized Schirmer tear test I

(STT1) was performed in another group of 10 chickens. Overall, PFL and the secretion of the aqueous phase of the tear film increased with age. Mean mSTT1 results were 5.00 ± 1.83 mm and 10.45 ± 2.58 mm for 5- and 42-d-old chicks, respectively. Mean PRCTTT results were 12.37 ± 1.80 mm and 25.58 ± 4.8 mm for 5- and 42-d-old chicks, respectively. Mean EAPPTT results were 7.13 ± 0.72 mm and 12.03 ± 0.92 mm for 5- and 42-d-old chicks, respectively. Mean STT1 for 42-d-old chicks was 11.40 ± 2.60 mm. Results obtained with endodontic paper points showed the lowest coefficient of variation and investigators found the test easier to perform. These values reported for broilers at different ages may be applied in poultry ophthalmology to help the diagnosis of lacrimal production disorders and to detect early disease symptoms that could cause economic losses in poultry production.

Key words: chick, tear production, normal parameters, ophthalmology

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INTRODUCTION

According to the United Nations, world population is expected to reach 9.8 billion in 2050 (UN, 2017). Improving poultry health is a key component in the prevention of a food shortage (Pimentel, 2004). Active prevention and promotion of health in poultry production includes the diagnosis of disease at an early stage before an outbreak occurs (Gyles, 1989; Pimentel, 2004). Some of these diseases cause large economic losses and manifest an increase of lacrimal production due to inflammation of ocular and respiratory tissues. Examples of such diseases are Newcastle and Marek's diseases, reoviruses, salmonellosis, and aspergillosis, as

well as poor management conditions that promote excessive ammonia production including poor ventilation (Calnek, 2001; Miles et al., 2006; Feitas Neto et al., 2007; Zande and Kuhn, 2007; Arné et al., 2011; Malliselo and Nkonde, 2015).

Three eye structures are responsible to produce the complex composition of the lacrimal film that covers the entire ocular surface. These structures are called goblet cells, Harderian gland (also known as the gland of the nictitating membrane), and lacrimal glands. Goblet cells produce the mucine layer which is responsible to establish the lacrimal film, while Harderian and lacrimal glands produce the aqueous layer which is responsible to lubricate and nourish the eye surface (Kern and Colitz, 2013). Meibomian glands which are modified sebaceous glands have not been described in the lids of any avian species (Murphy et al., 1990). The aqueous fraction of the lacrimal film can be measured by simple and quick methods in the majority of domestic animals. In birds, however, the small size of the eye makes this

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The use of sulfur hexafluoride microbubbles for contrast-enhanced ocular ultrasonography of the *pecten oculi* in birds

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Abstract

Background: The *pecten oculi* is a vascular and pigmented structure localized within the posterior segment of all avian eyes. Its primary function is not fully understood yet.

Objective: An ultrasonography (US) is a useful imaging modality for evaluation of the *pecten oculi*, the objective of this study was to investigate the utility of an intravenous contrast solution of sulfur hexafluoride (SF₆) microbubbles as a means of enhancing visualization of the *pecten oculi* in normal birds.

Animals studied: Ten adult individuals of the following avian species were evaluated: 1 roadside hawk (*Bubo magister*), 1 screech owl (*Asio scyllax*), 2 striped owls (*Asio flammeus*), 2 burrowing owls (*Athene cunicularia*), 2 ring-necked pheasant (*Phasianus torquatus*), and 2 domestic chickens (*Gallus gallus domesticus*).

Procedure(s): After baseline ocular sonograms were obtained in sedated animals, 4.5 µg/kg of a contrast solution containing SF₆ microbubbles was administered intravenously and US of the right eye was immediately performed. US was continued during injection to provide real-time imaging of the *pecten oculi* during vascular perfusion of contrast material. **Results:** Within 2–3 seconds following intravenous contrast administration, microbubbles reached the *pecten oculi* of all birds investigated and provided significant ultrasonographic contrast enhancement. **Conclusions:** SF₆ microbubble contrast ultrasonography in birds is a safe and easy procedure that provides increased contrast and enhanced visualization of the *pecten oculi*. Future use may enable further discovery of its physiologic functions and aid in the development of therapeutic plans for avian intraocular disease.

KEYWORDS

avian, diagnostic imaging, eye, ophthalmology, retina

1 | INTRODUCTION

The avian eye has several distinctive anatomical and physiologic features when compared to the eyes of other vertebrate taxa.¹ A large variability in eye shape, mechanisms of both lenticular and corneal accommodation, variable

numbers and positions of retinal specializations, and the presence of the *pecten oculi* are among the most conspicuous features.^{1–5} The *pecten oculi* is of particular interest as there is currently no consensus for its most important physiologic role.^{6,8} It is a pigmented and highly vascularized intraocular structure projecting anteriorly from the optic

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WINDOW COLLISIONS BY BIRDS IN THE SOUTH OF BRAZIL: EPIDEMIOLOGIC FACTORS, RADIOGRAPHIC AND NECROSCOPY FINDINGS
Journal of **Avian** Medicine and Surgery

Dear Dr. Fabiano Montiani-Ferreira,

I am pleased to tell you that your work has now been accepted for publication in Journal of **Avian** Medicine and Surgery.

It was accepted on 07/27/2020

Comments from the Editor and Reviewers can be found below.

Thank you for submitting your work to this journal.

With kind regards,

Dr. Thomas N Tully, Jr
Science EditorThomas N Tully, Jr, DVM, MS, Dipl ABVP (**Avian**), Dipl ECZM (**Avian**)
Science Editor
Journal of **Avian** Medicine and Surgery

Comments from the Editors and Reviewers:

Journal of Avian Medicine and Surgery
**WINDOW COLLISIONS BY BIRDS IN THE SOUTH OF BRAZIL: EPIDEMIOLOGIC
FACTORS, RADIOGRAPHIC AND NECROSCOPY FINDINGS**
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Abstract:	Birds are among the most visually adept group of animals on the planet, however their inability to visualize and discriminate translucent glass structures results in hundreds of millions of deaths worldwide due to high-speed collisions. Despite reports of avian glass collisions in North America, few studies have been developed in order to better understand this scenario in South America nor evaluate radiographic and necropsy findings of affected birds. Data from 100 cadavers were examined radiographically and by necropsy, while 186 collision reports were analyzed for seasonality. A total of 34 different species of birds within 22 families were reported, with <i>Turdus rufiventris</i> (12), <i>Zenaida auriculata</i> (12) and <i>Columbina talpacoti</i> (10) being the most common species. Only seven (20%) migratory species were reported: <i>Chaetura meridionalis</i> , <i>Elaenia parvirostris</i> , <i>Florisuga fusca</i> , <i>Pitangus sulphuratus</i> , <i>Sporophila caerulescens</i> and <i>Turdus amaurochalinus</i> . Males (51) were more frequently reported than females (5), and 50.1% of the males had active gonads on necropsy. The most common radiographic lesion, noted in 19.5% of the animals, was loss of coelomic definition, suggestive of hemorrhage. Prevalent necropsy findings included skull hemorrhages (77.3%), encephalic contusions (64.4%) followed by coelomic hemorrhages (40.7%). Most collisions (61, 32.8%) occurred during spring, the most common breeding season of avian species in Brazil. Cranioencephalic trauma was identified as the most reported cause of mortality in glass collision accidents. Migration does not seem to be the main predisposing factor for window collisions by birds in South America. Increased activity and aggression related to breeding season, especially in males, may be a more important predisposing factor for window collisions accidents.

1 WINDOW COLLISIONS BY BIRDS IN THE SOUTH OF BRAZIL: EPIDEMIOLOGIC
2 FACTORS, RADIOGRAPHIC AND NECROSCOPY FINDINGS.

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13 accidents or bringing dead animals that suffered these accidents.

14 **Author contributions:**

15 Gabrielle Adad Fornazari: Performed the experiment, collected data, conducted the research,
16 wrote the paper, developed methods

17 André Saldanha: Substantially edited the paper, helped to collect data

18 Rogerio Ribas Lange: Analysed the data

19 Tilde Froes: Contributed substantial materials, analysed the data

20 Daniel Klem Jr: Substantially edited the paper, analyzed the data

21 Bret A. Moore: Substantially edited the paper, analyzed the data

22 Fabiano Montiani-Ferreira: Conceived the idea, supervised research, edited the paper,
23 formulated hypothesis