

Submitted: 01/11/2017

Accepted: 03/09/2018

Published: 28/09/2018

Tear production and intraocular pressure values in clinically normal eyes of whooper swans (*Cygnus cygnus*)

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Abstract

The objective of this study was to determine the normal range of tear production and values of intraocular pressure (IOP) in clinically normal eyes of whooper swans (*Cygnus cygnus*). A total of 86 birds: 81 cygnets (6-10 weeks old) and 5 moulting – adult swans (3-6 years old) were examined in this study. Both eyes of all birds underwent a complete ophthalmic examination, including evaluation of tear production with the phenol red thread test (PRTT) and the assessment of the IOP by rebound tonometry, employing the TonoVet[®] device. The mean ± standard deviation of PRTT values were as follows: all cygnets (81) 22.59±3.48 mm/15s, male cygnets (45) 22.64±3.54 mm/15s, female cygnets (36) 22.53±3.44 mm/15s. IOP was as follows: all cygnets 11.30±3.55 mmHg, male cygnets 10.93±3.56 mmHg, female cygnets 11.74±3.50 mmHg. No statistically significant differences between right and left eye and between female and male cygnets were found for IOP and PRTT values. This study provides the reference values for the PRTT and IOP in healthy whooper swan cygnets, showing that PRTT determination of the tear production and rebound tonometry to evaluate the IOP methods are practical methods for ophthalmic examination in swans.

Keywords: Eye, Intraocular pressure, Phenol red thread test, Tear production, Whooper swan.

Introduction

There are seven swan species in the world (Brazil, 2003) and three of them are common in Latvia. One of these, the whooper swan (*Cygnus cygnus* L.) was found to breed only in North Russia and Scandinavia. Nowadays the breeding population of whooper swans has expanded its habitat southwards and its breeding population has become more stable by increasing in number in southern Scandinavia and the Baltic States (Haapanen, 1989; Axbrink, 1999). The species has been breeding in Germany, Denmark, Ukraine, Belarus, Hungary and the Netherlands (Abramchuk *et al.*, 2003; Grell *et al.*, 2004; Gaschak, 2005; Bauer and Woog 2008; Van Dijk *et al.*, 2010).

The first nesting whooper swan in Latvia was found in 1973 (Baumanis, 1975), but by 2007 the population had reached about 200 pairs (Boiko, 2008).

The whooper swan is listed in the International Union for Conservation of Nature (IUCN) Red Data Book (IUCN, 2017) and the EU Birds Directive, Appendix II). There are difficulties in performing a proper examination in birds with ocular diseases brought into veterinary clinics for treatment and rehabilitation with the aim of re-releasing healthy treated birds into the wild because of the limited diagnostic methods and knowledge of physiological ocular parameters of the healthy swan eye. Therefore, it is important to establish

normal ophthalmic parameters for the exotic animals such as wild birds. In the literature, normal values of ocular diagnostic tests have been published for domestic and wild animals (Ofri *et al.*, 2002; Montiani-Ferreira *et al.*, 2008; Lange *et al.*, 2012), and lately in water birds, ducks, and geese (Mood *et al.*, 2016).

Data are limited for many species, and there are currently no data available for swans. Standard ophthalmic diagnostic tests such as assessment of the intraocular pressure (IOP) with rebound tonometry and tear production with the phenol red thread test (PRTT) are very important to provide a complete ophthalmic examination in birds (Stiles *et al.*, 1994; Holt *et al.*, 2006; Jeong *et al.*, 2007; Storey *et al.*, 2009; Barsotti *et al.*, 2013).

The purpose of this study was to determine the physiologic values of tear production in clinically normal whooper swans, using the PRTT and IOP measurement using the rebound TonoVet[®] tonometer.

Material and Methods

The field study was performed near the most important ringing sites for cygnets and adult non-breeding birds, namely the fish ponds in Skrunđa (56°42'N, 21°59'E), Renda (57°04'N, 22°17'E) and Rimzāti (56°58'N, 22°10'E) in Latvia.

The study was performed fully respecting the welfare of the birds involved and the ethical criteria under

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which the bird identification ringing was performed. The research has been approved by the ethics committee of the Latvia University of Life Sciences and Technologies. Birds were restrained in a specifically designed Velcro jacket thus immobilizing the birds' body, feet, legs, and wings, with no pressure exerted on cervical region (Fig. 1). The neck was held in by the hand because whooper swans do not show any aggression and specific precautions are not needed. Bird capture, fixation, and ringing were performed according to internationally accepted guidelines (FAO, 2007).

A physical examination was performed before the ocular examination, and any animals with indications of systemic diseases were excluded from our study. An examination was performed by DVM, Dr.med.vet., an associate professor, specializing in veterinary ophthalmology. All birds included in this study were examined to ensure they were ophthalmologically healthy. Ocular examination included direct ophthalmoscopy (Keeler Practitioner, Windsor, UK), monocular direct ophthalmoscopy (PanOptic® ophthalmoscope, Welch Allyn, Romford, UK), slit lamp biomicroscopy (Kowa SL15, Nagoya, Aichi, Japan), and fundus photography (SmartscopeVet2® fundus camera, Sonomed Oy Ltd, Finland). A total of 86 birds, 81 healthy wild cygnets (45 males and 36 females) and 5 healthy adult birds (2 males and 3 females) were examined in this study. Sex and age was identified by the researcher group according to physiological and anatomical features (Demongin, 2016). The exact age of the birds was unknown, but cygnets (Fig. 2) ranged between 6-10 weeks of age and adult birds (Fig. 3) were between 3-6 years. The birds were classified into groups, differentiated by age and gender: all cygnets, male cygnets and female cygnets respectively and adult birds. To measure tear production, the PRTT was employed, using a single lot from one manufacturer (Tianjin Jingming New Technological Development Co., Ltd.). To perform the PRTT the non-toothed Wangenstein tissue forceps were used to facilitate placement of the 3-mm indentation at the end of the thread into the inferior conjunctival sac for the 15 seconds. After the removal of the test, the length of the strip for which the color changed from yellow to red was immediately measured in millimetres. All tonometric measurements were performed by the same person employing rebound tonometry with the Tonovet tonometer (TonoVet®, Tiolat Ltd. Finland) in the (d) calibration setting, with automatic calibration as provided by the device. Each measurement recorded was the automatically generated average after five successive readings. Measurements were taken between incursions of the nictitating membrane and care was taken not to apply pressure to the neck or periocular region.



Fig. 1. Whooper swans restrained in a specifically designed Velcro jacket.



Fig. 2. Whooper swan cygnet.



Fig. 3. Whooper swan adult (Moulting birds).

Statistical methods

Statistical analysis of the data was performed using statistical software programs SPSS and Microsoft Excel. Arithmetic mean values (\bar{X}) and standard deviation (SD) of the PRTT and IOP results were obtained from each eye separately. Normality was tested using the one-sample Kolmogorov-Smirnov test.

A paired sample t-test was used to compare the PRTT obtained from the right and left eye. An independent sample t-test was used to compare the mean PRTT and IOP values for bird's age and sex. P values less than 0.05 were considered to be statistically significant.

Results

The PRTT threads were placed without difficulty facilitated by the use of the forceps. The birds tolerated all the tests well. All numeric data obtained for PRTT test and IOP values in the population used in this study were normally distributed according to the Kolmogorov-Smirnow test. Measurements of PRTT in all 86 birds showed a mean \pm SD of 22.5 \pm 3.63 mm/15s in both eyes, as presented in Table 1, with a median of 23 mm/15s in the right and left eyes, showing no significant differences between eyes ($P>0.05$). For cygnets, the mean PRTT value was 22.59 \pm 3.48 mm/15s in both eyes of, with no difference in cygnets between the right and left eye measurement (Table 1). The mean PRTT in male cygnets for both eyes was 22.64 \pm 3.54 mm/15s, whereas the mean PRTT in females was 22.53 \pm 3.44 mm/15s respectively. Comparison between mean PRTT values in male and female birds showed no significant difference ($P>0.05$). For the adult bird group (5 birds) the average of PRTT values was 21 \pm 5.57 mm/15s in both eyes. The PRTT results in all bird groups showed no significant difference between PRTT values in birds of different age and gender and between right and left eye ($P>0.05$).

IOP in all 86 birds showed a mean \pm SD of 11.19 \pm 3.48 mmHg in both eyes with a median of 10 mmHg in the right and left eyes (Table 2), and no significant differences between left and right eyes ($P>0.05$). IOP mean values in cygnets was 11.30 \pm 3.55 mmHg both eyes. Mean IOP in male cygnets in both eyes was 10.93 \pm 3.56 mmHg, whereas in female cygnets were 11.74 \pm 3.50 mmHg. In the adult bird group (Table 2), the average of IOP values was 9.5 \pm 1.27 mmHg in both eyes, showing no significant differences in IOP values between both eyes ($P>0.05$), but values were significantly lower than in cygnets ($P<0.05$). The IOP results in all bird groups showed no significant difference between birds of different age and sex ($P>0.05$).

Discussion

This study provides reference ranges for PRTT and IOP in normal eyes of whooper swans, mainly relating to cygnets. Establishing normal ranges in healthy birds is very important to avoid diagnostic misinterpretations during ophthalmic examination and this is a relevant in swans as in other avian species.

To the authors' knowledge, PRTT and IOP values have not previously been reported in swans. Both tests are relatively easy to perform, however, active third eyelid movements can make it difficult to keep the PRTT thread in the lower conjunctival sac.

Table 1. Phenol Red Thread test values for the whooper swans.

Groups	No. of birds	Mean PRTT \pm SD (mm/15s)		
		Right eye	Left eye	Both eyes
All birds	86	22.35 \pm 3.56	22.35 \pm 3.72	22.5 \pm 3.63
Adult birds	5	20.6 \pm 5.22	21.4 \pm 6.5	21.0 \pm 5.58
All cygnets	81	22.46 \pm 3.44	22.72 \pm 3.54	22.59 \pm 3.48
Male cygnets	45	22.58 \pm 3.48	22.71 \pm 3.63	22.64 \pm 3.54
Female cygnets	36	22.30 \pm 3.43	22.75 \pm 3.47	22.53 \pm 3.44

Table 2. Intraocular pressure values for the whooper swans.

Groups	No. of birds	Mean IOP \pm SD (mmHg)		
		Right eye	Left eye	Both eyes
All birds	86	11.26 \pm 3.35	11.13 \pm 3.63	11.19 \pm 3.48
Adult birds	5	9.4 \pm 0.54	9.6 \pm 1.81	9.5 \pm 1.27
All cygnets	81	11.38 \pm 3.41	11.22 \pm 3.70	11.30 \pm 3.55
Male cygnets	45	11.02 \pm 3.44	10.84 \pm 3.71	10.93 \pm 3.56
Female cygnets	36	11.81 \pm 3.37	11.67 \pm 3.68	11.74 \pm 3.50

We found the PRTT to be as an appropriate test to use for tear production measurement in these birds because of the small size of the eye, eyelid fissure and it has shown restrictions when used in small eyes (Willis and Wilkie, 1999; Müller *et al.*, 2010). Also with the shorter test time period taken for this test that the Schirmer tear test (STT) and the lower level of tear production compared to the dog or cat in which the STT is widely used (Holt *et al.*, 2006; Storey *et al.*, 2009; Barsotti *et al.*, 2013).

All measurements were performed at approximately the same time of the day (9.00 am -12.00 pm) because of the possible diurnal variation in tear production and IOP (Beech *et al.*, 2003; Hartley *et al.*, 2006) and by the same examiner with the bird held in the same body position to minimize variations in restraint or study technique.

Data for wild animal species mostly concerns mammalian groups but we considered it inappropriate to compare our data with mammals, therefore we identified similar studies investigating other avian species. So far evaluation of normal tear production in large Psittaciformes like the cockatoo, macaw, Amazon, African grey, Eclectus and Pionus species showed mean PRTT test values 19.8 \pm 4.3 mm/15s in the right eye and 20.1 \pm 3.9 mm/15s in the left eye, somewhat lower than the values for the swans in the current study (Holt *et al.*, 2006; Storey *et al.*, 2009; Barsotti *et al.*, 2013) but they do have smaller eyes than

those of the swans investigated here. These large Psittaciforme birds were studied in northeast United States where the mean temperature in summer is an average of 80°F (26.8°C) and relative humidity is 71% (Holt *et al.*, 2006), compared to Latvia where mean temperature in summer is 64°F (17.8°C) and relative humidity is 76% (Latvian Environment, Geology and Meteorology Centre). These differences may relate to differences in environment quite as much as ocular anatomy or physiology. Previously Barsotti and colleagues have shown tear production also in Eurasian Tawny owls, Little owls, Common buzzards, and European kestrels (Barsotti *et al.*, 2013), however, we used the PRTT test instead of STT.

A previous study has shown that after positioning of threads in the fornix of upper and lower eyelids, the PRTT values of the common Mynah birds were 17.5±3.1 mm/15 s and 19.2±2.5 mm/15s, respectively, showing a significant difference between PRTT values for upper eyelids and lower eyelids (Rajaei *et al.*, 2015). In our study, we placed PRTT in the lower eyelid and results show 22.59±3.48mm/15s in the cygnet group and 22.5±3.63 mm/15s in all-bird group showing somewhat higher results than the current study. These differences might be explained by the differences in lifestyle - the Common Mynah (*Acridotheres tristis*) is an omnivorous living open woodland bird but the whooper swan is a water bird.

In research in geese and ducks, which are similar to swans in both their ocular anatomy and the environment in which they live, the Schirmer tear test was used to evaluate tear production showing values of 6.2±2.2 mm/min in ducks and 5.5±2.6 mm/min in geese, but these data are difficult to compare to those in the present study because of the different methods of evaluating tear production (Mood *et al.*, 2016).

Regarding measurement of avian IOP, a study in Screech owls determined the mean IOP to be 9±1.8 mmHg measured with TonoVet “d” calibration but 11±1.9 mmHg measured with TonoPen-KL, showing lower IOP with TonoVet (Harris *et al.*, 2008) while measurement of IOP with the TonoPen mean values varied between 9.83±3.41 mmHg in little owls and 8.53±1.59 mmHg in European kestrels to 17.2±3.53 mmHg in Common buzzards (Reuter *et al.*, 2011). The IOP values in our study lie between these two values at 11.19±3.48 mmHg measured with TonoVet tonometer. Another study suggested that the rebound tonometer possibly overestimates IOP values in diurnal raptors and underestimate IOP values in nocturnal raptors but the variations from the manometric value in these birds was small and varied between species (Reuter *et al.*, 2010, 2011). Considering the differences in the present study between young and adult birds, a study in three week old chickens, showed IOP values of 17.51±0.13 mmHg, that did not differ significantly with broiler and

layer chickens lines (Prashar *et al.*, 2007), but again it should be noted that these are not water birds. In one study of captive black-footed penguins (*Spheniscus demersus*) tonometry showed a much higher IOP (31.77±3.3 mmHg) than in our study (Gonzales-Alonso-Alegre *et al.*, 2015), and a later publication of ocular findings and reference values in penguins showed IOP measured with the Tonovet tonometer “d” calibration to be 29.1±7.16 mmHg in Macaroni and 24.1±5.09 mmHg in Rockhopper penguin, both higher than in swans evaluated here (Bliss *et al.*, 2015). A more recent research study on IOP in ducks and geese showed the average IOP to be 10.2±2.2 mmHg in ducks and 9.1±2.0 mmHg in geese, measured with TonoVet tonometer (calibrated at the “d” setting as in the current study) giving results similar to those in the present study (Mood *et al.*, 2016).

To conclude, the investigation of clinical parameters obtained in this study will help veterinary ophthalmologists and wild animal veterinarians to evaluate changes in tear production and IOP in swans more accurately to help further in the treatment of wild birds. Further study of the diurnal variation of the physiological parameters like tear production and IOP should be undertaken and is planned by the current authors.

Conflict of interest

The authors declare that there is no conflict of interest.

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