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Research article

Ex vivo analysis of ultraviolet radiation transmission through ocular media and retina in select species

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ABSTRACT

The aim of this study was to assess the transmission of the ultraviolet (UV) radiation (200–400 nm) through intact enucleated globes of different species (dogs, cats, pigs, rabbits, horses, and humans) using spectrophotometry. Globes of cats (n = 6), dogs (n = 18), pigs (n = 10), rabbits (n = 6), horses (n = 10), and humans (n = 4) were analyzed. A 5–10 mm circular area of sclera and choroid from the posterior aspect of the globe was removed under a surgical microscope, leaving the retina intact in all species except the horse. Glass coverslips were added in horses and rabbits due to retinal and globe fragility. The %T of wavelengths from 200 to 800 nm were measured through the ocular media (cornea, aqueous humor, lens, and vitreous humor) and retina, and compared between species. The globes of cats and dogs allowed the most amount of UV radiation transmission, while those of pigs and humans allowed the least amount of UV radiation transmission. A small amount of UV radiation transmission through the ocular media was detected in the rabbit and horse. Results from this study will support further vision research that may be used to train companion, working, and service animals.

1. Introduction

The mammalian visual range is approximately 400–700 nm and many species are equipped with protective mechanisms to reduce or prevent ultraviolet (UV) radiation (<400 nm) from entering parts of the eye and damaging cellular structures, particularly the retina (Dillon et al., 1999, 2000; Douglas and Jeffery, 2014; Peichl, 2005). For example, in humans and other primates, the lens blocks out almost all UV radiation due to the presence of the metabolite 3-hydroxykynurenine glucoside (3-HKG) (Dillon et al., 1999). Humans who are aphakic have reported to see light in the UV spectrum because 3-HKG no longer filters out UV wavelengths (Dillon et al., 2000). In most species, the cornea does not transmit significant amounts of UVB (<315 nm) and UVC radiation (<280 nm) due to nucleic acid composition (Douglas and Jeffery, 2014). Additionally, the presence of retinal oil droplets in certain bird species prevents UV radiation from being processed into a visual signal (Douglas and Jeffery, 2014). Furthermore, lack of UV vision may enhance spatial resolution and visual acuity (McDonald et al., 2020; Yovanovich et al., 2020a). Wavelengths in the UV spectrum can contribute to chromatic aberrations as shorter wavelengths are more refractive, leading to lower visual acuity (Gouras and Ekesten, 2004; Nordling, 2019). Thus, species that rely on high visual acuity such as humans, benefit from decreased to absent UV radiation transmission to lessen chromatic aberrations (Douglas and Jeffery, 2014; Yovanovich et al., 2020a).

In contrast, evolution has resulted in other vertebrates such as birds, fish, amphibians, reptiles, and rodents to detect light in the UV spectrum to aid with foraging, mate selection, predator detection, and various other tasks for survival (Bowmaker, 2008; Hogg et al., 2011; Jacobs et al., 1993; Martin et al., 2015; McDonald et al., 2020; Mitchell et al., 2021; Peichl, 2005). Peak absorption of the UV-sensitive type of the short-wavelength sensitive photopigment (SWS1) is approximately 360 nm and conserved across many species, including birds, fish, amphibians, reptiles, and rodents (Yokoyama and Shi, 2000). Animal communication for foraging and mating in the UV wavelengths is most common between 360 and 400 nm (McDonald et al., 2020), and UV radiation

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detection may enhance texture recognition in some species (Yovanovich et al., 2020a). For example, arctic reindeer have an extended visual range into the UV spectrum which helps with foraging behavior and detection of predators where ice and snow reflect large amounts of UV radiation (Hogg et al., 2011). Rodents use UV radiation to detect conspecific urine marking in their environment for communication (McDonald et al., 2020) and birds of prey can find rodent-rich areas by scanning the ground for these urine markings (Viltala et al., 1995). Even though many animals have protective mechanisms to reduce UV exposure to the retina, the ability to detect UV light has been beneficial to certain species. These potential benefits could extend into companion and working animals, such as dogs, cats, and horses.

Dogs are dichromats with two spectral classes of cone visual pigments (S-cones and M/L-cones). The short-wavelength-sensitive cones (S-cones) have their peak sensitivity at 429 nm and medium-to long-wavelength sensitive cones (M/L-cones) have peak sensitivity at 555 nm (Jacobs, 1992; Neitz et al., 1989) with the variation of sensitivities between individuals within the same canine species being small (Jacobs et al., 1993). Although characterized by their peak sensitivity, the spectral range of cone visual pigments is not fully uniform. In addition to the α-band range in the visible part of the spectrum where their sensitivity peaks, visual pigments are also sensitive to shorter wavelengths, the β-band (Govardovskii et al., 2000). The importance and degree of the absorbance in this range is not fully understood. However, the result is a sensitivity overlap between different classes of visual pigments: the β-band sensitivity of the M/L-cone opsins overlaps with the peak of the S-cone visual pigments, while the β-band sensitivity of the cone visual pigments stretches into the ultraviolet spectrum (Jacobs, 1992; Ramon et al., 2009). This suggests that the cone visual pigments in the species studied are likely to be sensitive to UV-wavelengths.

To our knowledge, the transmittance of UV radiation through intact ocular media, including the retina, has not been evaluated in the dog, cat, horse, rabbit, pig, or human. A recent study has demonstrated that humans can behaviorally respond to UVB radiation (Hammond and Renzi-Hammond, 2018). We considered evaluation of the whole globe through intact ocular media and retina to be essential to evaluate the direct transmission of UV radiation as we are primarily interested in the wavelengths of light that reach and pass through the retina. Estimating the transmission of UV radiation through the ocular media and retina is the first key component to determine if an animal has the potential to see UV wavelengths. The current research was designed to reduce the use of live animals while attempting to emulate a living model.

2. Materials and methods

2.1. Preparation of whole eyes

All eyes used in this study were donated. All animals were treated in accordance with the ARRIVE guidelines and ARVO Statement for the use of animals in Ophthalmic and Vision research. Animals were obtained from various sources such as shelters, veterinary hospitals, and laboratories, and euthanized for reasons unrelated to this study. Eyes were enucleated from dog, cat, horse, rabbit, and domestic pig cadavers in a standard transconjunctival approach, while leaving a small amount of periorbital tissue at the equator for handling. Estimated or known ages of the animals and humans in this study are depicted in Supplementary Table 1. Following enucleation, animal eyes were enclosed, kept on ice or refrigerated, and analyzed between 2 and 24 h of death, with the exception of 5 pig eyes which were measured within 48 h of death. This was done to minimize the effects of decomposition and corneal opacity. Eyes with any ocular abnormalities, such as corneal vascularization or edema, lens pigmentation, or cataracts, were excluded. Presence of nuclear sclerosis was recorded.

Donated human eyes were obtained from the Miracles in Sight Eye Bank, which provides eyes multiple times per year to North Carolina State University (NCSU) researchers. Eyes with clear corneas without cataracts were requested and used. Ocular cooling was initiated within 1–2 h of death, and human eyes were kept within moist chambers without fixatives. The shortest time from death to analysis was 3 days and the longest was 7 days.

Preparation and dissection of the eyes were completed under a surgical operating microscope (ZEISS, Triangle Instruments Company, Durham, NC, USA). All the corneas were intact and were irrigated with balanced salt solution during dissection and measurement to keep them moist. The orientation of the eyes was determined based on the location of the optic nerve and long posterior ciliary arteries (Gelatt, 2021). Either a 5 mm (rabbit), 8 mm (horse and cat) or 10 mm (dog, pig, and human) disposable punch biopsy (Robbin's Instruments, Houston, TX, USA) was used to outline and dissect the posterior scleral and choroid dorsal to the optic nerve. Punch biopsy size was estimated based on the size of the globe and fragility of the retina; larger punch biopsies were selected for larger globes. Once the punch biopsy was to the level of the choroid, a 2.5 mm angled spoon blade (Ambler Surgical, Exton, PA, USA) and corneal section scissors were used to remove the sclera from the choroid in a level plane (Fig. 1A). The spoon blade was then used to perforate the choroid layer at the lateral edge of the punch biopsy site. The retina was visible at this step, protruding through the choroidal defect. Right and left corneal section scissors were used to gently cut away the remaining choroid in a circumferential fashion leaving the retina intact (Fig. 1B).

One exception to this method of dissection and punch biopsy size choice occurred in the horse. The horse eyes were more challenging to dissect while keeping the retina intact as vitreal back-pressure perforated the retinas in all cases. Therefore, a smaller punch biopsy (8 mm) relative to globe size was selected and full-thickness biopsies were created in all horse globes. Following, a glass coverslip (Cover Glass, Corning Inc., Corning, NY, USA) was placed over the opening and fixed to the sclera with glue (Super Glue, Loctite®, Westlake, Ohio, USA) to prevent leakage and/or prolapse of the vitreous which may impact the results. Rabbits also had very fragile retinas that tore easily after choroid removal, and therefore a glass coverslip was placed over the retina in rabbit globes to prevent tearing of the retina and bulging of the vitreous.

2.2. Spectrophotometry

A spectrophotometer (Cary 50 UV–Vis Spectrophotometer, Varian, Inc., Australia) with associated scanning software was utilized to transmit and record light at wavelengths ranging from 200 to 800 nm. The UV–Vis scan rate was set to 600 nm/min and the UV–Vis data interval was set to 1 nm. The beam size for spectrophotometry was 1.5 mm × 1 mm. The spectrophotometer was operated in dual-beam mode and set to baseline before each daily session and zeroed before each eye to ensure accurate recordings. Accuracy in wavelength and transmission reported by the spectrometer are verified by measuring optical standards including laser line pass filters, edge pass filters, and neutral density filters.

Eyes were imaged on an adjustable stage (CALIDAKA, London, UK) with an overlying piece of Styrofoam for pinning to adjust positioning inside the spectrophotometer (Fig. 1C). Two 22 g needles were used to pin the eye utilizing periorbital tissue to prevent movement during measurement. The stage and secured globe were placed within the spectrophotometer after removing the microcell holder. Eyes were aligned so that the scleral opening faced the light source present at one side of the spectrophotometer and the cornea was in line with the detector present on the opposite side. The corneas were irrigated immediately prior to measurements to simulate a live animal as closely as possible.

Before recordings were made, proper alignment within the spectrophotometer using the align program with fixed wavelength of 500 nm was performed for each eye, which measured the percent of transmitted light through the globe. The highest percent alignment was attempted for each eye and recorded. Once optimal alignment was achieved, the

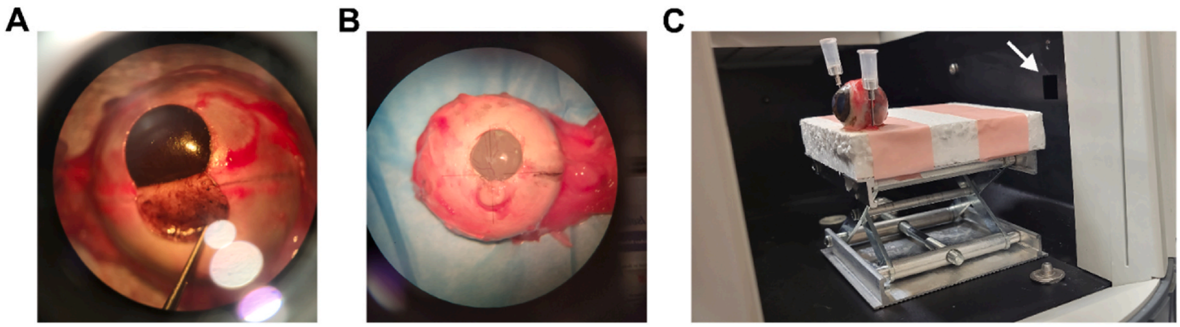

Fig. 1. Dissection and spectrophotometry of cadaver globes
(A) Pigmented choroid of a pig globe is exposed following dissection of connective tissue and sclera; (B) Translucent retina of a pig globe is exposed following dissection of the choroid; (C) Globes were pinned on to an adjustable stage with an overlying piece of Styrofoam. The stage and secured globe were placed within the spectrophotometer and aligned so that the scleral opening faced the light source (arrow) present at one side and the cornea was in line with the detector present on the opposite side.

scan program was initiated with an open spectrophotometer and percent transmission (%T) was recorded for each wavelength between 200 and 800 nm. For the purposes of this study, 800 nm was set at 100% T to allow for comparison between all runs. For inter-species comparison, %T was measured once per globe for each species, except in humans where %T was measured four times per globe.

To assess the effect of the coverslip, retina, and lens on transmission, one globe from a single cat was analyzed as.

1. Globe with a coverslip and intact retina
2. Globe with a coverslip but no intact retina

While the other globe from the same cat was analyzed as.

3. Globe with an intact retina but no coverslip
4. Lens separately

Each of the above globe variations and the lens were measured four times in the spectrophotometer. An individual coverslip was also placed in the spectrophotometer for measurement. Globes without an intact retina and coverslip were excluded from this study due to vitreal prolapse at the biopsy site. To assess intra-class correlation coefficient (ICC) associated with spectrophotometry measurements, a single globe of a dog, cat, horse, rabbit, and pig was measured 10 times (placed in and removed from the spectrophotometer for each scan).

Five outcome measures were calculated and assessed: (1) Ratio of UV radiation transmission, (2) %T, (3) %T cut off point, %50 T, and (4) %UVA transmitted. Ratio of UV radiation transmission is a ratio of the area under the curve from 200 to 400 nm (UV spectrum) and area under the curve from 200 to 800 nm (total light spectrum analyzed). The %T is the average %T at select wavelengths from 200 to 800 nm. The %T cut off point is the wavelength at which %T reaches <1%. The %50 T is the wavelength at which %T reaches 50%. Lastly, %UVA transmitted is a measure of the proportion of light between 315 and 400 nm (Douglas and Jeffery, 2014).

2.3. Statistical analysis

Statistical analysis of ratio of UV radiation transmission was performed with computerized statistical software GraphPad Prism for Mac v9 (GraphPad Software Inc., La Jolla CA, USA). This ratio was compared via Brown-Forsythe and Welch one-wave ANOVA. Comparison between the right vs. left eye in the same animal, and presence of nuclear sclerosis were assessed in the dog and horse using Wilcoxon matched-paired *t*-test and Welch's unpaired *t*-test, respectively. Significance was set at $p \leq 0.05$. Based on data from a preliminary study and an assumption of an approximate normal distribution, we achieve at least 80% power for inter-species comparisons with 4 eyes per species and 90% power with 6 eyes per species using the Wilcoxon rank sum test. Statistical analysis of ICC was performed with the *irr* package using the *icc* function in R (version 3.2.3) (Gamer et al., 2019). Reliability of ICC was considered excellent if it measured ≥0.9 (Koo and Li, 2016). Data is presented as mean with 95% confidence interval (CI).

3. Results

3.1. Animals

The number of eyes obtained differed between species based on availability. Eyes included in this study were dogs (n = 18), cats (n = 6), horses (n = 10), rabbits (n = 10), pigs (n = 10), and humans (n = 4). Mild to moderate nuclear sclerosis was present in 6/18 dog, 4/10 horse, and 4/4 human eyes (Fig. S1).

3.2. UV radiation transmission

The ratio of UV radiation transmission through the ocular media and retina of cats was the greatest when compared to dogs ($p < 0.0011$), pigs ($p < 0.0002$), and humans ($p < 0.0001$) (Fig. 2). Explicitly, the ratio of UV radiation transmission through the ocular media and retina of cats was 2.1 times higher than in dogs, 21.0 times higher than in pigs, and 20.3 times higher than in humans. The ratio of UV radiation

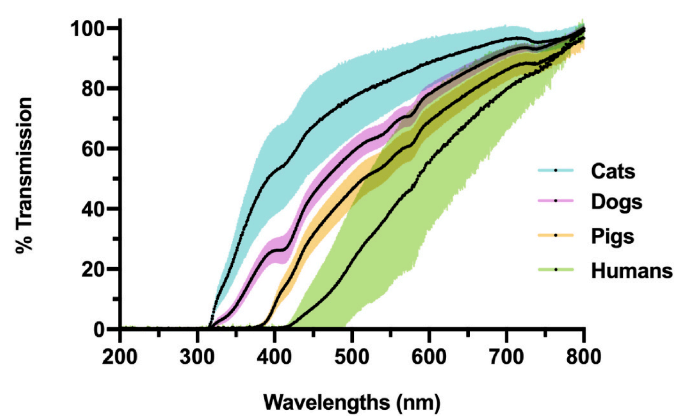

Fig. 2. Spectral transmission through the ocular media in select species
Average spectral transmission (%T) of cats (n = 6), dogs (n = 18), pigs (n = 10), and humans (n = 4) from 200 to 800 nm. A 95% CI is demonstrated by color shading surrounding each species line. Cats transmitted the most UV radiation compared to dogs ($p < 0.0011$), pigs ($p < 0.0002$), and humans ($p < 0.0001$). Amount of UV radiation transmission was compared using Brown-Forsythe and Welch one-wave ANOVA.

transmission was also significantly greater in dogs in comparison to pigs ($p < 0.0001$) and humans ($p < 0.0001$); specifically, 9.9 and 9.6 times higher when comparing dogs to pigs and humans, respectively. There was no significant difference in the ratio of UV radiation transmission between pig and human eyes ($p = 0.997$).

Further transmission data of select species are presented in Table 1. Notably, the ocular media and retina of cats and dogs transmitted the highest percentage of UV radiation with 52.5% and 26.1% transmission at 400 nm, and 33.3% and 12.0% at 360 nm, respectively, and the %50 T was 435 and 466 nm, respectively. The UV transmission in pigs and humans was 7.8% and <1% at 400 nm, respectively, and the %50 T was 485 and 585 nm, respectively. UV radiation transmission through the ocular media and retina of rabbits and horses was at 22.0% and 25.4% transmission at 400 nm, respectively (Fig. 3), and the %50 T was 468 and 435 nm, respectively.

The presence of a coverslip was taken into consideration when interpreting cumulative UV radiation transmission. In two eyes from a single cat, the presence of the coverslip covering the exposed retina significantly decreased the transmission of UV radiation by 31.3% when comparing the globe with an intact retina and coverslip to the fellow globe with an intact retina but no coverslip support ($p < 0.0001$). The presence of the retina also significantly decreased transmission of UV radiation by 7.4% when comparing a globe with an intact retina and coverslip to a globe with coverslip but no retina ($p < 0.0001$). Intact ocular media (cornea, aqueous humor, lens, and vitreous) and retina resulted in significantly decreased transmission of UV radiation by 14.2% when comparing a globe with an intact retina but no coverslip to the lens separately ($p = 0.0001$). Analysis of the lens alone in the single cat revealed that the lens is the main determinant factor of the %T cut off point with successive addition of ocular structures, such as the retina and coverslip, decreasing the area under the curve from 200 to 800 nm. Interestingly, %T through the coverslip alone in the spectrophotometer does not reveal in any decrease in visual or UV radiation transmission (Fig. 4).

3.3. Right vs. left eye and nuclear sclerosis

Comparison of the right vs. left eye in same animal was completed in 9 dogs and 4 horses. In the paired analysis, there was no significant difference between the right and left eyes in both dogs ($p = 0.73$) and horses ($p = 0.88$).

The effect of nuclear sclerosis on the ratio of UV radiation transmission through the ocular media and retina was examined in two species: dogs and horses (Fig. 5). In dogs, there was a significant difference between normal vs. eyes with nuclear sclerosis ($p < 0.0082$), with UV transmission of normal eyes being 1.6 times higher than that of eyes with nuclear sclerosis. In horses, there was no significant difference in UV radiation transmission between normal vs. eyes with nuclear sclerosis ($p = 0.1$). Age-related yellowing or brunescence of the lens was not noted in any eyes in these species.

3.4. Intraclass correlation coefficient

Intraclass correlation coefficient (ICC) was used to assess if globe placement and alignment would affect repeatability of the spectrophotometry data. The ICCs for all species analyzed, dog, cat, horse, rabbit, and pig, revealed values ≥ 0.9 indicating excellent repeatability even when the globe was placed in and removed from the spectrophotometer for each of the 10 scans.

4. Discussion

In the present study, a simple method for measuring UV radiation transmission through intact ocular media and retina has been described. As the globes were positioned at a distance from the aperture to the detector, we can only report the direct transmittance, whereas the total transmittance, including scattered light, would have been higher (Boettner and Wolter, 1962). From the species studied, cats and dogs allowed the most amount of UV radiation transmission, while pigs and humans allowed the least amount of UV radiation transmission. With the presence of a coverslip in the rabbit and horse, and absence of retina in the horse, only a small amount of UV radiation transmission was allowed in these species.

The differences in UV radiation transmissions between species observed in this study are most likely attributed to lifestyle and geographic distribution (Yovanovich et al., 2020b). These two factors determine the amount and spectral composition of light an animal is exposed to, both in the temporal (day and night) and spatial dimensions (latitude, elevation, and habitat type) (And et al., 1997; Caldwell et al., 1980; Roberts, 2012). Therefore, ocular structures likely evolved to 'match' the light environment in which a visual system performs, with nocturnal species possessing highly transmissive ocular structures to maximize the number of photons that reach the retina (Bowmaker, 2008; Jacobs et al., 1993; Peichl, 2005; Yovanovich et al., 2020a) and diurnal species filtering out short-wavelength radiation to preserve spatial resolution and visual acuity, and prevent radiation damage (Collier and Zigman, 1987; Douglas and Jeffery, 2014; Walls and Judd, 1933). Nocturnal species were not included in this study. However, when comparing crepuscular (cats, dogs, and rabbits) versus diurnal (domestic pigs and humans) species, our study supports higher transmission of UV radiation in crepuscular species. Crepuscular animals are active primarily during the twilight period, compared to diurnal species, animals for whom light is an 'unlimited' resource. Horses are largely diurnal (Hall et al., 2018) with feral horses being diurnal and crepuscular (Dobbie et al., 1993; Groves, 1989; McCourt, 1984), and thus horses had similar UV radiation transmission data to domestic pigs and humans.

Anatomic variations between species can additionally play a role in UV transmittance through the eye. For example, the size of the lens can affect UV transmittance with larger lenses blocking more short-wavelength radiation as evidenced in horses, alpaca, oryx, and anoa. On the other hand, smaller lenses, such as those found in cats, dogs,

Table 1
Summary of transmission data of select species from 200 to 800 nm.
(1) %T: average %T at select wavelengths from 200 to 800 nm, (2) %T cut off point: wavelength at which %T reaches <1%, (3) %50 T: wavelength at which %T reaches 50%, and (4) %UVA transmitted (Douglas and Jeffery, 2014): a measure of the proportion of light between 315 and 400 nm, are presented for each species.

Species %T	800 nm	400 nm	380 nm	360 nm	340 nm	320 nm	300 nm	%T Cut off Point (nm)	%50 T (nm)	%UVA Transmitted (Douglas and Jeffery, 2014)
Cat n=6	99.2	52.5	45.3	33.3	18.4	4.58	0.09	314	435	30.3
Dog n=18	99.9	26.1	21.0	12.0	4.69	0.99	0.09	319	466	12.3
Rabbit n=10	98.6	22.0	5.91	0.37	0.17	0.17	0.09	367	468	4.24
Equine n=10	100.1	25.4	1.28	0.05	0.03	0.03	0.02	378	435	2.90
Pig n=10	96.5	7.77	0.83	0.21	0.15	0.08	0.07	381	485	1.01
Human n=4	100.0	0.60	0.76	0.29	0.51	0.50	0.41	415	585	0.46

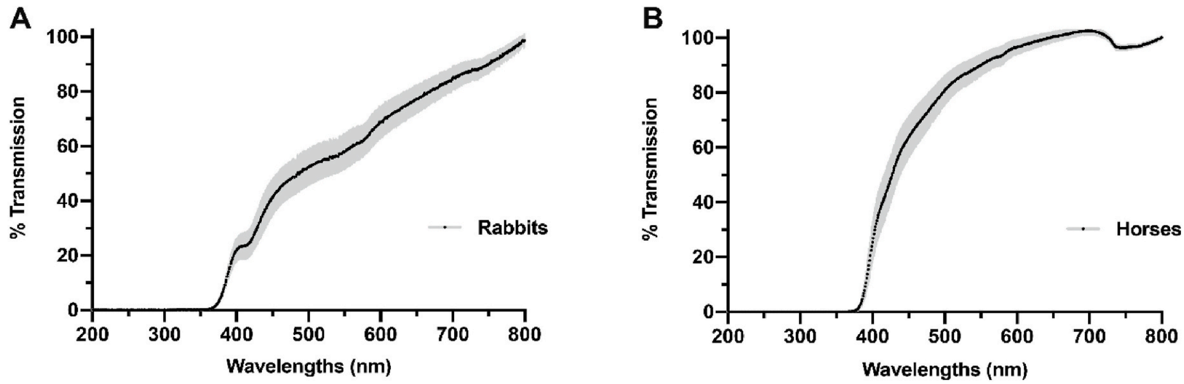

Fig. 3. Spectral transmission through the ocular media in the rabbit and horse.
Average spectral transmission of (A) rabbits with coverslip and intact retina (n = 6), and (B) horses with coverslip and no retina (n = 10). A 95% CI is demonstrated by gray highlighting surrounding each species line. Both the rabbit and horse globes were assessed with coverslips due to fragility of the retina. The rabbit and horse globes transmitted less than 1% of radiation at wavelengths below 367 nm and 378 nm respectively and ratio of UV radiation transmittance were 4.24% and 2.90% respectively.

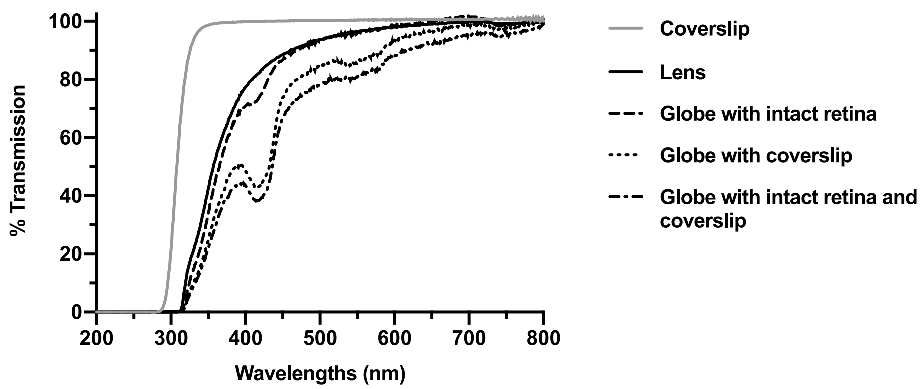

Fig. 4. Comparison of transmission through various ocular media of a cat globe and glass coverslip
Average spectral transmission of a (1) coverslip, (2) lens, (3) globe with an intact retina but no coverslip, (4) globe with a coverslip but no intact retina, (5) globe with a coverslip and intact retina. Globe samples were from a single cat.

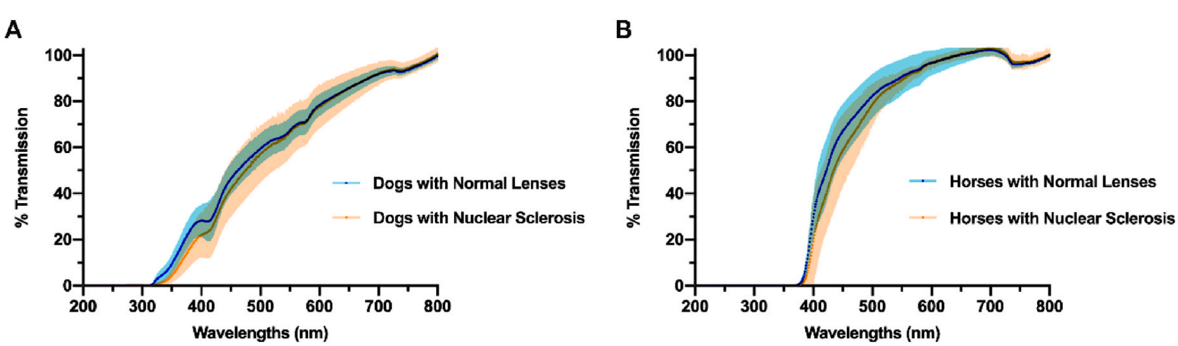

Fig. 5. Effect of nuclear sclerosis on spectral transmission in the dog and horse
Average spectral transmission of (A) dogs without (n = 12) and with (n = 6) age-related nuclear sclerosis, and (B) horses without (n = 6) and with (n = 4) age-related nuclear sclerosis. A 95% CI is demonstrated by color surrounding each line. Dogs with nuclear sclerosis transmitted significantly less UV radiation ($p < 0.0082$), while there was no significant difference in UV radiation transmission between normal vs. eyes with nuclear sclerosis in horses ($p = 0.1$). The presence of nuclear sclerosis were assessed in the dog and horse using Wilcoxon matched-paired *t*-test and Welch's unpaired *t*-test, respectively.

rabbits, and rodents, have the capacity to transmit more short-wavelength radiation to the retina (Douglas and Jeffery, 2014).

The tapetum lucidum is a cellular layer within the choroid present in some vertebrates (e.g. cats, dogs, and horses) and invertebrates, that improves mesopic and scotopic vision by acting as a mirror to reflect light that was not absorbed by photoreceptors in the retina during the first pass and returning it to the photoreceptors, increasing the probability that additional light photons will be absorbed by photopigment (Ollivier et al., 2004; Schwab et al., 2002; Shinozaki et al., 2013; Walls, 1967). As light passes through the retina more than once in these species, UV transmission through the retina was evaluated in this study. The role of the tapetum lucidum in UV vision has not been studied extensively and was not evaluated in this study as the pigmented choroid was removed to allow light to pass through translucent to clear structures of eye from the spectrophotometer light source to the detector. Animals with a tapetum lucidum are reported to have higher levels of UV vision due to tapetal reflectance (Hogg et al., 2015; Nordling, 2019). For example, in deep diving hooded seal, the tapetum lucidum preferentially reflected UV radiation ten times more than other wavelengths (Hogg et al., 2015).

Life span and age-related changes may furthermore affect UV radiation transmission (Boettner and Wolter, 1962). UV radiation can be damaging to cellular structures, and when cumulated over time, may result in more significant effects such as retinal phototoxicity (van Norren and Vos, 2016). Thus, animals with shorter lifespans, such as cats, dogs, rabbits, and rodents may be able to tolerate UV radiation, but those same dosages could be detrimental to animals with longer lifespans, including humans, primates, domestic pigs, and horses (Gouras and Ekesten, 2004) leading to the evolution of protective mechanisms in the latter species. Nuclear sclerosis, the age-related compression of older lens fibers leading to increased density of the center of the crystalline lens, may also affect UV radiation transmission in the eye by inducing light scattering, thus reducing transparency and the number passable light rays (Sigelman et al., 1974). In our study, dogs with mild to moderate nuclear sclerosis had a significantly decreased ratio of UV radiation transmission compared to dogs without nuclear sclerosis. Graphed data in the horses demonstrated a trend of decreased UV radiation transmission in eyes with mild to moderate nuclear sclerosis; however, statistical significance was not reached in this species. This discrepancy may be due to a smaller sample size in horse compared to dog eyes. Several studies define a relationship between short-wavelength radiation and the formation of cataracts (Anduze A.L., 1993; Cruickshanks et al., 1992; Sasaki et al., 2003; Taylor, 1989). Previous studies in human lenses have demonstrated that younger lenses transmit more UV radiation (Sliney, 2016), although the transmission of UV even in young adult primates is low (Dillon et al., 2000). It seems nuclear sclerosis, though a degenerative product of aging, may play a role in blocking harmful short-wavelength radiation in older animals or longer living animals.

Previous studies have evaluated the transmittance of UV radiation through various separate (Boettner and Wolter, 1962; Douglas and Jeffery, 2014; Hogg et al., 2015) and combined parts of the eye (Dillon et al., 2000) in different species. Upon comparison, our results were most similar to studies that examined combined parts of the eye. Dillon et al. (2000) assessed light transmission through an intact cornea, aqueous humor, and anterior vitreous complex, and found in rabbits that wavelengths shorter than approximately 350 nm did not reach the retina with a 2% transmittance down to 320 nm. Similarly in our study, %T in the rabbit reached <1% at 367 nm with the slight discrepancy most likely attributed to the presence of the posterior vitreous, retina, and coverslip. The Commission Internationale de L'Eclairage (2012) report provides reference transmission and absorption data for human eye research. Upon comparison, the report's direct transmittance data through clear ocular media of a 70-year-old human eye is parallel to the results of our human eye data of 71 and 77-year-old donors (Fig. S3).

On the contrary, disparities were noted between our results and studies that examined single ocular structures. Douglas and Jeffery (2014) measured %UVA transmittance, a measure of the proportion of light between 315 and 400 nm UVA radiation, through lenses in a variety of species with values in the cat, dog, rabbit, horse, and wild pig lens being 58.9%, 61.3%, 12.7%, 4.6%, and 43.6%, respectively. These values were higher compared to our %UVA values through combined ocular media, which were 30.3%, 12.3%, 4.24%, 2.90%, and 1.01% in the cat, dog, rabbit, horse, and domestic pig, respectively. In addition to the current study measuring direct transmittance through multiple intraocular structures, this may be due to difference in subspecies or breed, aging effects including addition of globes with nuclear sclerosis in the dog and horse groups, or different spectrophotometer equipment and alignment between the light beam and optic axis of the eye. However, while the lens is the structure that determines the amount of UV incident on the retina in most all vertebrates (Douglas and Marshall, 1999), loss due to reflection of normally incident radiation at the interface between air, cornea, aqueous humor, lens, vitreous, and retina should also be taken into consideration (Boettner and Wolter, 1962). Additionally, lens transmission is highly variable with the lens in some species allowing almost as much UV radiation as the cornea, while in others it can remove all UV (Douglas and Marshall, 1999). Boettner and Wolter (1962) collected data separately from aqueous humor, lens, vitreous, and retina from human cadavers, computed the successive transmittance radiation through the whole eye, and showed a decrease in direct transmission of wavelengths from 300 to 1200 nm. Similarly, our cat globe with intact retina vs. cat lens data revealed a significant decrease in UV radiation transmission by 14.2%. This supports that intact ocular media and retina should be considered when assessing UV radiation transmission through the eye. Assessment of the lens alone leads to an overestimation on how much UV radiation reaches the photoreceptor outer segments in the retina, and furthermore, an animal's visual capabilities in the UV range.

There were several limitations to this study. An attempt was made to limit the amount of time between death and analysis, but this was not consistent and the time gaps from post-mortem to measurement could have affected the transmittance of light through the ocular structures. In particular, the human eyes used in this study had a significantly longer interval from death to analysis. However, our human data was still similar to the reported human reference transmission data from the Commission Internationale de L'Eclairage (2012), thus allowing direct comparisons even to freshly excised animal eyes. Another limitation was the fragility of the retina during dissection in the horses and rabbits. Multiple attempts were made to dissect to the level of the retina in a few horses, however the pressure of the vitreous tore the retina seconds after the choroid was removed, which is most likely due to the large volume of vitreous in the horse or age-related vitreal degeneration. Rabbits also had a very delicate retina, but the retinas were preserved for measurement with the help of coverslips. Other limitations were achieving maximal alignment in the spectrophotometer and variation in light transmission when comparing eyes and species. Even with careful but extensive manipulation of the globe and adjustable stage, alignment values varied between each globe in each species; however, those variations are within a range (Fig. S2). Interestingly, the ICC used to assess if globe placement and alignment would affect repeatability of the spectrophotometry data was excellent (≥0.9) in all species assessed. Thus, the variation in light transmission data may be a consequence of species variation in nucleic acids and structural protein components content of the ocular media and retina (Douglas and Marshall, 1999) or species variation in corneal and scleral rigidity (Gelatt and Gelatt, 2011). Additionally, the glass coverslip may not have always been directly perpendicular to the light beam while also being parallel to the cornea in the horse and rabbit eyes. This could have reflected some of the wavelengths before being detected by the sensor. This amount of reflection was likely negligeable due to the high %T measured with the glass coverslip alone. An integrating sphere was not available with the model of spectrometer used, however an optics simulation revealed that the lensing effect of the various sized eyes would not have a major effect on the data.

There are several next steps following this study. To assess the capacity for UV vision, assessment of electroretinography (ERGs) of live dogs using a Ganzfeld that emits LED UV light is needed (Powell et al., 2021). Further assessment of the behavioral component to UV vision could employ four-choice vision testing device or preferential looking tasks on dogs, which is commonly performed for models of retinal disease (Gearhart et al., 2008; Graham et al., 2019). Similar behavioral testing has been successful in fish (Powell et al., 2021). Knowledge about the capacity of vision in companion and working animals can aid our understanding on how they visually process their environments. Using a stepwise approach to evaluating UV vision, we may explore opportunities to use this knowledge in environmental enrichment and training.

5. Conclusion

In conclusion, cats and dogs allowed the most amount of UV radiation transmission, while pigs and humans allowed the least amount of UV radiation transmission and rabbits and horses were in the middle. Age-related nuclear sclerosis significantly decreased transmittance of UV radiation in canines, but significance was not found in horses. This research serves as the physical evidence of UV wavelengths reaching the retina in select species, serving as the basis for future UV vision research.

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Declaration of competing interest

There are no conflicts of interest to disclose for any of the authors.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exer.2023.109550.

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