Evaluation of retinal images for identifying individual dogs

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Objective—To find whether vessels in the ocular fundus changed over the lifetime of Beagles and whether any changes were substantial enough to likely preclude positive identification of individual dogs by use of their retinal vascular patterns.

Animals—18 Beagles.

Procedures—Fundic photographs of both eyes of 18 Beagles taken at 1 or 3, 5, and 7 or 9 years of age were digitalized. Photographs were analyzed by use of 2 software programs. One was used to determine vessel numbers and widths and the other to determine the locations of the 3 largest vessels. Measurements were compared over time periods in the life of each dog. Only observations made at baseline (1 or 3 years of age) and again at 5 and 9 years of age were included in the statistical analysis, as these points were common to all dogs.

Results—No significant changes in numbers or locations of the blood vessels were detected over time. Widths of the vessels decreased significantly as the dogs aged.

Conclusions and Clinical Relevance—The ocular fundus of Beagles changed over each dog’s lifetime in that the retinal blood vessels became smaller but did not change in number or location. Results suggest that digitalized retinal images can likely be used to identify dogs over their lifetimes. (Am J Vet Res 2006;67:2042–2045)

Positive identification of individual dogs is important for veterinarians, dog breeders, and animal rescue agencies. Unalterable identification of dogs via hip joint radiography and certification by the Orthopedic Foundation of America, ocular identification and registration by the Canine Eye Registry Foundation, or semen collection would ensure that dog substitutions could not be made. Animal shelters would also benefit from a reliable and easily achieved means of animal identification.

Identification methods presently used in dogs include use of morphologic features, tattooing, and SC placement of microchips. Identification by morphologic features is an inexact means of identification because many dogs have similar appearance. Tattooing of dogs in the groin area or the auricular pinna provides a more objective means of identification, but subsequent readings of tattoos can be difficult because of fading of the tattoo ink as well as growth of hair over the tattooed area. In addition, tattoos can be altered or removed.

Microchip placement is also an objective means of identification but is not without problems. Placement of the chip requires SC injection over the shoulder-neck area with a large needle. This procedure could result in infection, or the microchip could be accidentally deposited in the thoracic cavity or lungs. The microchip can migrate from its site of insertion to many other sites (such as internal organs), which could cause associated problems as well as difficulty finding the microchip when identification by a microchip reader is attempted. Retinal morphology has been used as an accurate means of identifying individual humans. Identification of humans by the pattern of their retinal blood vessels was first suggested in 1935, and the first commercial product for human retinal blood vessel identification appeared in 1985. Retinal-scanning systems have been used in the military and in financial institutions in high-security access areas. A system for retinal imaging and identification of individual cows is presently in use in the United States.

Retinal imaging may be a valid means of individual identification in dogs because, as in cows and humans, there are numerous retinal vessels emanating from the optic disk. The larger vessels are veins, and the smaller vessels are arteries. These vessels form a distinct pattern unique to each individual. Although it has never been reported, it is assumed that retinal blood vessels do not change in pattern throughout the life of the animal and that retinal blood vascular patterns are virtually unalterable. Ocular fundic diseases could potentially alter retinal vessels by making them more tortuous or making them smaller; however, these conditions do not alter the positions of the vessels within the retina.

The purpose of the study reported here was to determine whether age-related fundic changes in clinically normal Beagles were of sufficient magnitude to preclude positive identification of individuals by use of digital retinal scanning.

Materials and Methods

Photographic slides of the ocular fundi of approximately 100 Beagles were obtained by one of the authors (ACL) during a long-term study of the effects of radiation on those dogs. Dogs chosen for the study reported here were nonirradiated control dogs. Ocular photographs of 18 dogs (11 sexually intact males and 7 sexually intact females) were selected on the basis of the clarity and focus of the photographs over the time periods intended for study. The photographs were scanned and digitalized.
The dogs were housed at Colorado State University during the years 1980 to 1990. The ocular fundi of the dogs were photographed annually during their entire lives or until 10 years of age. Photographs were taken with a fundus camera and 35-mm slide film. Camera settings were the same for all photographs taken. All dogs were placed on a table and manually restrained. Their pupils were maximally dilated with 1 drop of tropicamide.

All dogs remained healthy throughout the study period, and no ocular abnormalities were seen. To detect any age-related fundic changes that were substantial enough to preclude positive identification of individual dogs, 1 photograph of each eye from each dog at each age (1 or 3 years, 5 years, and 7 or 9 years) was analyzed.

Two software programs were used to examine the digitalized photographs and analyze the fundic vessel patterns in each dog. This was done to determine the number, widths (sizes), and positions (angles and lengths) of the large vessels present. To determine whether the numbers and sizes of the larger vessels in each dog's fundus changed substantially over time, each digitalized image was analyzed with software that measured the widths and counted the numbers of larger vessels by detecting them where they intersected 2 concentric circles projected around the optic disk (Figure 1). The starting point for plotting the vessels was a line drawn from the disk to the ora ciliaris retinae at the 3 o'clock position in the fundus; this represented 0°. The 2 concentric circles were moved synchronously and placed around the optic nerve to form a ring 0.2 mm wide. The center of the circles (denoted by a dot) was situated over the optic pit, and the placement of the rings was done by the same person each time. The program analyzed the digital image and filtered it by removing the high-frequency noise coming from the imaging device. This was done by taking a moving mean value in which the high-pass frequency was set by the window size of the moving mean value. The vessels were identified by segmentation, which is the process of separating features in an image by their contrast with the background (eg, the tapetum or areas of nontapetal pigmentation). Segmentation is based on a limit threshold in which the data are examined and a threshold value is selected that gives a distinct separation of the vessels from the background. Three width measurements were used to compute a mean value. All of the major vessels were detected, and their locations were plotted on a graph and counted according to their positions (in degrees around the circles) and mean widths (in pixels; Figure 2).

A second software program was used to determine whether the 3 major blood vessels on each of the retinal pictures changed in position over time. This program was used to compensate for rotation around a clockwise axis in the different photographs. With this program, the angles, from a horizontal line through the center of the optic pit, subtended by the 3 vessels as they crossed the border of the optic disk, were determined at each age (Figure 3). In addition, the distances from a point placed in the optic pit to points placed where each of the 3 vessels crossed the edge of the optic disk were calculated. These 4 points were identified in each photograph. The initial point (point 0) was manually located in the center of the optic pit. Points 1, 2, and 3 were located in the centers of the 3 largest blood vessels closest to the 9, 12, and 3 o'clock positions, respectively, at the points where they crossed the optic disk. The distance between point 0 and each other point was measured 3 times, and these values were used to compute a mean value. These data were defined as the distance data and reported in pixels.

Statistical analysis—For each dog, the number of vessels and the mean widths (in pixels) of the vessels were calculated at each time point. Mean widths were evaluated by taking a moving mean value in which the high-pass frequency was set by the window size of the moving mean value. The vessels were identified by segmentation, which is the process of separating features in an image by their contrast with the background (eg, the tapetum or areas of nontapetal pigmentation). Segmentation is based on a limit threshold in which the data are examined and a threshold value is selected that gives a distinct separation of the vessels from the background. Three width measurements were used to compute a mean value. All of the major vessels were detected, and their locations were plotted on a graph and counted according to their positions (in degrees around the circles) and mean widths (in pixels; Figure 2).

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Figure 2—Plot of the vessels on the fundus of a retina in Figure 1. The horizontal axis represents the vessel position in degrees, and the vertical axis represents their widths in pixels.
use of ANOVA for repeated measures (repeated in time). The frequency of vessels was evaluated by use of a generalized linear mixed-model method with an assumed Poisson distribution. The statistical model included side (ie, right or left eye), sex, time, and the 2- and 3-way interactions among these 3 factors, all as fixed effects.

For evaluation of the distance from point 0 and angles measured at points 1, 2, and 3, the initial value was used as a baseline value. Relative values (percentages) were calculated as baseline values divided by subsequent values multiplied by 100%. Results were used in an ANOVA appropriate for repeated measures to assess changes in time (ie, age). Each measurement was analyzed as a separate outcome. A multivariate ANOVA was used to evaluate the 3 outcomes simultaneously, with time as the only fixed effect. To maximize the power of the test, only values for which age was 5 or 9 were included. Values of $P < 0.05$ were considered significant.

Results

For all 18 dogs, all photographs from each individual dog's eyes at all time points were grossly similar (Figure 4). When the photographic slides were stacked on top of each other and compared, the shapes (eg, curves and branches) and numbers of blood vessels did not appear subjectively to change over time. However, the fundic image on the slides became noticeably less clear as each dog's age increased, which was attributed to changes in the optical media (eg, the cornea, lens, and vitreous) and did not affect the ability to easily delineate the blood vessels. The diameter of the blood vessels appeared to decrease considerably as each dog aged, which was confirmed by the measurement data. Mean width of vessels decreased significantly over time (1 year of age, 11.70 pixels; 5 years of age, 10.33 pixels; and 9 years of age, 6.98 pixels). Neither sex nor side was associated with this change. A significant sex effect was detected for the number of vessels per dog when both eyes were considered. Female dogs had fewer vessels (15.19/dog), compared with male dogs (20.12/dog). Age and side were not associated with the number of vessels per dog. Retinal hemorrhages were evident in one 9-year-old dog; these did not change the ability to measure the retinal vessels, so the dog was included in the study.

Age was not significantly associated with changes in distance from point 0 for points 1, 2, or 3 (Table 1). However, multivariate ANOVA revealed that age was

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Table 1—Mean ± SD (percentage of baseline [age, 1 year]; range) values of the distances (in pixels) from the center of the optic disk to the points at which each of 3 major vessels (points 1, 2, and 3) crossed the edge of the optic disk in Beagles at 5 and 9 years of age.

Figure 4—Photographic images of the retina of dog, obtained at 1 (A), 5 (B), and 9 (C) years of age. Notice a slight decrease in the widths of the vessels and a slight cloudiness of the image in C, although the numbers and locations of the vessels do not change with age.
significantly associated with change in the angle of the vessels measured at points 2 and 3, but not at point 1 (Table 2). This significant change did not seem intuitively correct, and the digital photographs, marked with the 4 points, were carefully examined visually. It was apparent that this statistical finding was an artifact because in all of the dogs, both points 2 and 3 shifted away from point 1 in the same direction by almost the same number of pixels. When the digital scans of the photographic slides of an eye of all dogs over the 3 age ranges were superimposed on a computer screen by manually aligning the centers of the optic pits and point 1, it became subjectively obvious that points 2 and 3 had indeed shifted. This was thought to be attributable to the axis at which the initial photograph had been taken rather than to an actual shifting of the vessel.

**Discussion**

The fact that the retinal vascular pattern of most mammalian species does not change substantially over time seems to be accepted by most ophthalmologists, although no data that support this concept could be found. In the study reported here, the fundus did not change enough with age to likely preclude positive identification of an individual. The artifact introduced by differences in the axis at which photographs are taken will need to be taken into account when developing a software program to analyze the angle data. It was interesting that although the numbers and positions of the retinal vessels did not change over time, vessel widths became smaller, which was probably associated with a generalized decrease in circulation as seen in organs and limbs because of aging.

Digital retinal imaging is presently being used to identify cattle, which is important in tracking diseases and settling ownership disputes. This technology could also be valuable as a simple and effective means of identifying dogs. Although eye diseases such as glaucoma, progressive retinal atrophy, and retinal degeneration could alter the vascular patterns enough to make them unrecognizable, these diseases are relatively rare in the general dog population. Cataracts and corneal opacities could decrease or eliminate the ability to visualize and photograph the fundus, but surgery or medical treatments could reverse the lesions enough to restore this ability. Results of the present study suggest that normal aging of Beagles will not significantly alter retinal vascular patterns and individuals might be identified by their vascular patterns throughout their lives. Although Beagles were used, it is likely that the results of this study can be applied to the other breeds and mixed-breed dogs.

**References**