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Biological Considerations Pertaining to Use of the Retinal Vascular Pattern for Permanent Identification of Livestock

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ABSTRACT: Our objective was to characterize the retinal vascular pattern (RVP) as a stable biomarker for use in identification of livestock and to characterize the ability of different users to collect images of RVP of cattle using prototype equipment specifically developed for that purpose. The central retinal artery and vein enter the eye along the optic nerve and divide to supply the retinal surface. The geometric configuration of this vascular bed develops and is completed during fetal growth. Other authors have reported that retinal angiogenesis is a Laplacian process which is ubiquitous in nature and follows branching patterns seen in rivers, trees, roots, and erosion channels. RVP images from livestock are converted into a quantifiable format using a digital camera. Indices are then created from the patterns resulting from a hashing function to allow rapid one-to-many searching. The number and position of branches, along with the diameter of each vessel combine to offer an information rich biomarker for use in animal identification. Digital images (n=625) of bovine RVP were obtained using a specialized camera. To characterize these images, the dominant trunk vessel of a subset (n=52) was positioned vertically and branches on the right and left of the trunk and other branching points were counted. Branches from the left (LB=6.4, 2.2; mean, variance) and right (RB=6.4, 1.5) of the vascular trunk; total branches from the vascular trunk (TBVT=12.8, 4.3), and total branching points (TBP=20.0, 13.2) showed differences across animals. A paired comparison of RVP from both eyes of 30 other animals confirmed that eyes from the same animal differ. RVP images of 4 cloned sheep from the same parent line were evaluated to confirm the unique RVP in genetically identical animals. The ability of 6 users (ranging from novice to advanced) to obtain RVP images was evaluated based on time required to obtain an image and the % of images obtained in <25 and <15 sec. There was a significant ($P<0.05$) user effect with advanced users surpassing novice. The uniqueness of the RVP, when coupled with GPS coordinates allows for the unalterable association of animal with owner and premises, thus improving the reliability of processes such as contaminated product recall, disease epidemic containment, and subsidy payment schemes.

Key Words: Retinal vascular pattern, Animal identification, Biomarker

Introduction

Source verification and identification of animals has long been a concern in the livestock industry. Recently, however, it has become an important global issue, attracting the attention of governments and regulatory agencies, food safety agencies, retailers and consumers. Verifying the source of livestock is important for food safety, continuous improvement in cooperative supply chain marketing programs, disease surveillance, fraud prevention in animal subsidy programs and country of origin labeling issues. Demand for secure identity preservations systems will continue as consumers, retailers, vertically integrated programs and governments expect dramatic improvements in all of these areas (Golden 1998).

The retinal vascular pattern is a biometric identifier. This is based on the premise of the uniqueness of each animal's retinal vascular pattern which is present from birth and does not change over the animal's life (Marchant, 2002). Simon and Goldstein (1936) reported that, in humans, every eye has a unique pattern of blood vessels. Later, this finding was further substantiated by Huntzinger and Christian (1978) in their study of identical human twins. Additionally, Prince et al. (1960) stated that the vascular pattern of the retina is very different from one species to another.

The objective of this study was to characterize the retinal vascular pattern (RVP) as a stable biomarker for use in identification of livestock. A secondary objective was to characterize the ability of different users to collect images of RVP of cattle using equipment specifically developed for that purpose.

Materials and Methods

Digital images (n=625) of the RVP of cattle were taken with hardware and software developed by Optibrand Ltd., LLC using a device known as an OptiReader™. The OptiReader™ device is a combination hand-held computer and ocular fundus digital video camera. The camera uses near infrared light to illuminate the ocular fundus and transmits full motion video at 15 frames per second to the hand held computer. The operator sees the full motion video on the computer's LCD display. Software evaluates and captures the first frame presented that fits the predetermined parameters of clarity and information.

The hand-held computer contains GPS satellite receiver board and antenna. The latitude and longitude, along with a satellite set time-date stamp, are encrypted and become part of the image record. This record also includes the controller device's CPU identification number.

To obtain RVP images, cattle were worked through a squeeze chute in a normal processing manner. The user approached the animal's head and directed the camera into the eye. The RVP images and time to acquire each image for each user were recorded for later analysis.

A subset (n=52) of these RVP images were further evaluated. Branches from the left and right of the vascular trunk; total branches from the vascular trunk and total branching points showed differences across animals. In addition, a paired comparison of RVP from both eyes of 30 other animals confirmed that eyes from the same animal differ. RVP images of 4 cloned sheep from the same adult parent material were evaluated to confirm the unique RVP in genetically identical animals.

Image acquisition data were analyzed using the PROC MIXED procedure of SAS with user as fixed and device and date as random effects. Least squares means and standard errors were computed accounting for unequal observations by user.

Results and Discussion

Woodward et al. (2001) defined a biometric identifier as "any measurable, robust, distinctive physical characteristic…that can be used to identify the claimed identity of the individual. Measurable means that the characteristic can be easily presented to a sensor and converted into a quantifiable, digital format. The robustness of a biomarker is a measure of the extent to which the characteristic is subject to significant changes over time. Distinctiveness is a measure of the variations or differences in the biometric pattern among the general population. The higher the degree of distinctiveness, the more unique the identifier".

The central retinal artery enters the inside of the eye along the optic nerve and then divides to supply the inner retinal surface (Hogan and Zimmerman, 1962). Correspondingly, the venous system drains from the inner retinal surface into the central retinal vein and exits the eye along the optic nerve. The geometric configuration (i.e. artery and vein size, shape, concentration, length, etc.) of this vascular bed development occurs during fetal growth as vasculogenesis (organization of cells to form blood vessels; Baldwin, 1996) and angiogenesis (the budding and branching of vessels from pre-existing vessels; Baldwin, 1996) occur.

The retinal vascular pattern in domestic livestock was described by De Schaepdrijver et al. (1989) to have the following characteristics: 1) the presence of a large vascular network in the major portion of the light sensitive portion of the retina; 2) blood vessels extend from the optic disk to the neighborhood of the jagged margin between the light-sensitive and light insensitive portions of the retina; 3) comprised of large and small retinal vessels; with the large arterioles near 100 µm and large venules near 200 µm making them readily visible.

Figure 1 depicts the RVP of a bovine collected with the OptiReader™. Note the distinct vertical trunk with branching vessels radiating from this trunk. Also note additional branching of the smaller vessels. The number and position of branches, along with the diameter of each vessel combine to offer an information rich biomarker for use in animal identification. Kinoshita and Honda (1991) reported that retinal angiogenesis is a Laplacian process which is ubiquitous in nature and follows branching patterns seen in rivers, trees, roots, and erosion channels.

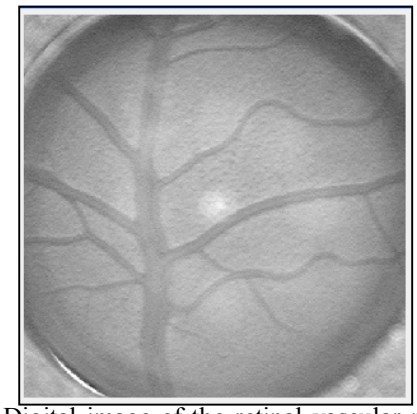

Figure 1. Digital image of the retinal vascular pattern of a bovine. This image depicts the branching and tortuous outline of the blood vessels that make each RVP distinct.

Table 1 contains data describing the mean and variance of vessel branching in bovine retina imaged with the OptiReader™. Images from the subset of 52 random individuals illustrate the large amount of information available for uniquely identifying individuals using RVP. Very large orders of combinations to distinguish animals reliably result from combining information about the relative positions of branch vessels, angles of branching and size of the branches. When number of branching points is coupled with position, diameter and proximity of vessel patterns, an almost infinite number of distinct information indices can be characterized. In addition, RVP images from both eyes of 30 animals were characterized and are shown in Table 1.

Table 1. Mean and variance of branching points in digital images of retinal vascular patterns from cattle.

	Mean	Variance
Random individuals (n=52 cattle)		
Left branches	6.4	2.2
Right branches	6.4	1.5
Total branches from the vascular trunk	12.8	4.3
Total branching points in the image	20.0	13.2
Paired eye images (n=30 cattle)		
Left branches	5.2	1.8
Right branches	5.2	1.8
Total branches from the vascular trunk	10.4	4.8
Total branching points in the image	16.5	14.3

Figure 2 depicts the RVP image from two sheep that are clones of the same adult parent material. Note the

diversity of vessel patterns in these images. Observationally, there appears to be no more similarity between these clones than there is between unrelated sheep. Further analysis is required on larger numbers of images to test this hypothesis.

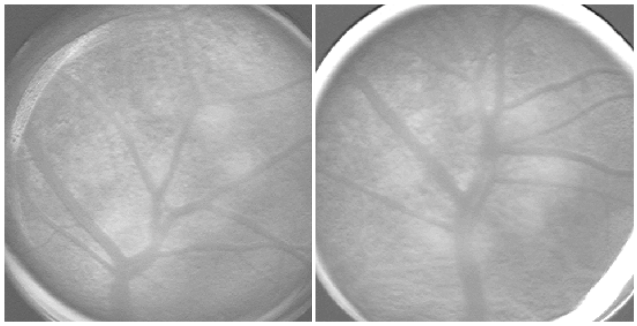

Figure 2. Retinal vascular patterns from 2 ovine clones produced from the same adult parent material.

Table 2 shows image acquisition data from six users using the OptiReader™. There was a significant ($P<0.05$) user effect in time to acquire images, the percent of images obtained in <25 and <15 sec. It does not appear that acquisition of images would add appreciably to the time required to process cattle. However, training and experience are important components for use of the OptiReader™ to successfully collect RVP images for animal identification.

Table 2. Least Squares Means of time required to acquire images by six different users using the OptiReader™.

USER	n	Time to Acquire Image*, Sec		Images Acquired in <25 Sec*, %		Images Acquired in <15 Sec*, %	
		LSM	SE	LSM	SE	LSM	SE
1	81	41.7	6.1	56.3	7.5	35.5	8.8
2	40	24.7	8.8	71.2	10.0	58.0	10.7
3	279	25.8	3.9	67.7	5.0	42.8	5.8
4	169	27.9	4.4	69.2	5.5	50.7	6.5
5	10	64.6	15.0	24.2	15.7	7.1	17.1
6	46	93.9	7.9	28.2	9.1	20.1	10.8

*Significant effect of user ($P<0.05$).

Another component employed in the Optibrand™ system is use of GPS technology to mark each image with time, date, and location, and the data storage and retrieval software. This method allows individual animals to be identified unambiguously, with an extremely high degree of distinctiveness (Shadduck, 1999).

Implications

The RVP of livestock is an information rich biomarker that meets the criteria of uniqueness outlined by Woodward et al., (2001). The process of obtaining RVP images appears to be compatible with other comparable livestock management practices. Using the RVP as part of a secure source verification process for livestock provide a humane, fraud-resistant tool for many applications in food production from livestock.

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