THESIS

STRENGTHENING IDENTIFICATION OF HIGH –RISK ANIMALS USING A NOVEL IDENTIFICATION APPROACH

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY SANDRA P. PORTER ENTITLED: STRENGTHENING IDENTIFICATION OF HIGH-RISK ANIMALS USING A NOVEL IDENTIFICATION APPROACH BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

STRENGTHENING IDENTIFICATION OF HIGH –RISK ANIMALS USING A NOVEL IDENTIFICATION APPROACH

The need for animal disease surveillance is a subject of constant discussion within the United States. Choosing an appropriate method of identification and trace-back that coincides with commerce is of the utmost importance. The production cycle of the imported feeder animal is fairly well defined: animals enter the U.S., are either sent to stocker or to feedlot operations, and after finishing are sent to the abattoir. The lifecycle of the imported roping animal is not clearly defined; the animals enter the U.S. and eventually the food chain, with limited knowledge of movements between importation and harvest. A novel form of animal identification utilizing retinal imaging was tested to maintain animal identification during the production cycle of both Mexican roping steers and spayed feedlot heifers. This secure and reliable method of identification combines GPS capabilities with the vascular pattern of the ocular fundus. Incorporation of this technology into a lifetime trace-back system for high risk animals that has the capability to follow animals through their production cycle to the abattoir when other forms of identification are lost. To test the ability of this technology to maintain animal identification 102 spayed feeder heifers were retinal imaged at the time of importation. The feeder heifers were verified at the feedlot and again at harvest. Nine hundred thirty five recreational animals were retinal imaged at the time of importation. Recreational animals typically have a lifespan longer than feeder cattle, and due to the scope of the research, were only followed for the first 90-120 days in the U.S. Recreational animals have been pinpointed as a high-risk source of cattle tuberculosis (Mycobacterium bovis) within the US, and maintaining animal identification throughout the production chain would benefit the U.S. in the event of an animal disease outbreak. This technology has the capability to bridge the gap between a Mexican identification system that allows herd of origin trace-back and the U.S.

In order for the technology to be successful, new operators must be able to be trained to efficiently and capture retinal images in a timely manner that can be used for identity re-establishment. A field trial was conducted to compare the performance of two novice operators to an expert operator. Operator performance was measured by the time required to capture a retinal image and the match comparison score when an operators images were compared.

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CHAPTER I

Objective of Thesis

STRENGTHENING IDENTIFICATION OF
HIGH–RISK ANIMALS USING A NOVEL IDENTIFICATION APPROACH

Field Trials:

- Determine if the Optibrand System can be used to maintain animal identification through the production chain for Mexican imported spayed feeder heifers.

- Determine if the Optibrand System can be used to maintain animal identification for Mexican imported recreational animals through the importation process and as they are distributed through recreational channels.

Operator Comparison:

- Evaluate the ability of newly trained OptiReader operators to capture quality retinal images that can be used for identity re-establishment.

CHAPTER II

INTRODUCTION

In 2005, 22 of 35 bovine tuberculosis cases reported in the United States were linked to animals with official Mexican ear tags at the time of slaughter. Five other cases indicated that the infected cattle originated from Mexico. An additional five cases were linked to a 'M' branded, chronically infected steer in a hospital pen in a small feedlot. Upon further investigation, it was determined that 22 cases in feedlot cattle with Mexican import ear tags could be traced to herds in the following Mexican states: Durango (7), Chihuahua (4), Nuevo Leon, Coahuila, Tamaulipas and Veracruz (2 each) and finally Jalisco, Aguascalientes and Campeche with one case each (Meyer, 2005).

In 2006, 27 of 28 bovine tuberculosis cases were deemed to be from fed steers or heifers, considered to be beef animals. One of the 27 had been used for roping, i.e. 'recreational type-activities'. The animal was imported in February 2004 at the Presidio, Texas port-of-entry in a lot of 59 animals. Between February 2004 and November 2005 the animal was used for recreational activities in Kansas and Oklahoma before moving to a Kansas ranch for finishing. At the Kansas location, the steer exposed 104 Brangus breeding cattle on the ranch to bovine tuberculosis. After further investigation, the Brangus cattle were depopulated, and the exposed cattle were appraised to be worth $82,480. The breeding cattle owners did receive federal indemnity. Neighboring herds and any herds that potentially were exposed were also tested. At report time, no other herds had been affected by the single steer. Further investigations were ongoing to track the remaining 58 potentially exposed animals. (Orloski and Meyer, 2006).

These cases were found using the slaughter surveillance method at Federally Inspected Processing Facilities. The United States relies heavily on the skill and diligence of Food Safety Inspection Services (FSIS) and Animal and Plant Health Services (APHIS) to monitor animal health. In a 2002 survey of FSIS inspectors from a variety of plants, one-third of respondents expressed concern that the "current system in place would not be sufficient for a major animal disease outbreak" (FSIS, 2002). The inspectors are referring to a trace-back system that would allow investigators to efficiently find the herd of origin in the event of an animal disease crisis.

The threat of bovine tuberculosis is a matter of great concern to the United States. Bovine tuberculosis (*mycobacterium bovis*) was responsible for the spreading of tuberculosis (M. bovis) to humans through un-pasteurized milk (Lo Bue, 2006). While processing milk products greatly reduced the number of human tuberculosis cases caused by *M. bovis*, *M. bovis* can be transmitted to humans from cattle by inhalation. This direct contact spreading is reason to keep surveillance high, as abattoir workers, veterinarians and veterinary students are all at risk (Lo Bue, 2006). The majority of technologically advanced countries do not have human problem with *M. bovis.* This paper will address the evaluation of a novel form of animal identification and potential trace-back system for a high risk population of animals. The need for a secure, tamperproof form of identification is becoming more clear as the U.S. consumer and foreign markets take a larger interest in the systems that are in place for animal verification.

Chapter III
Review of Literature

HESI A BTRCAETKNRNWIKNGday, consumers are taking more interest in the foods they consume than in the past. This is evident by the increase in the number of products claiming credence attributes i.e. "organic", "natural", "hormone free" and other marketing programs designed to meet the consumer's desire for accountability and choice in the grocery store. This change is due to the fact that there is a heightened awareness of food-related safety issues among today's consumers combined with a more educated public (Sparks, 2002). Consumers not only want assurance that the animal was treated humanely, but also that it was fed correctly, and produced in an environment as close to 'natural' as the consumer can envision. With recent occurrences of product recalls and food-borne illnesses, there is also a strong demand for source verification and supply chain identification of products in the United States. As there is increased publicity, attention and information (accurate and not) regarding E. coli, bovine spongiform encephalopathy (BSE) and other similar issues, it seems consumer eating habits are changing. In order to reinforce consumer confidence, a system for identifying, maintaining identification, and verifying animals from the farm to the fork is important. While other countries have established systems to do this, the United States is still in the process of formulating their plan of action for animal identification, traceability and trace-back.

The term 'traceability' is often referred to as the solution for identification and trace-back problems. Traceability is defined by the International Organization for Standardization

(ISO) as the "ability to trace the history, application or location of that which is under consideration." This vague definition gives little indication of what a system providing 'traceability' would actually entail. However, deciding what is 'under consideration' is a large challenge. The methods for managing the movements and processing of a cereal grain intended for a processed product would be very different than tracking the processing and fabrication of a carcass intended for whole muscle cuts. A system that would allow tracking of every input and process would require large investments and would likely be cumbersome for commerce. Instead of allowing one large-scale system to fit all needs, a better system would allow each industry/participant to dictate what their needs are and design a system that would meet those needs. Allowing each group to determine the breadth, depth and precision needed is likely to be more advantageous than a 'one size fits all' approach. Golan (2005) describes the breadth of a system as the amount of information that is collected, depth is how far back or forward the system tracks the relevant information and finally precision is defined as the degree of assurance with which the tracing system can pinpoint a particular food product's movement or characteristic. A traceability system for livestock will need a variety of components to be successful including: individual animal identification that can be carried through the production chain, standardized record keeping system and a method for searching records in the event animal health/food safety problem.

Benefits of Animal Identification. In 2005, a minimum of $82,480 (U.S.) in indemnity was paid out in response the tuberculosis threat due to one Mexican imported animal. This animal crossed the U.S.-Mexico border into Texas in a group of 59 animals. From September 2005 to August 2006 the Mexican state of Chihuahua exported 13,399 head of rodeo type stock to the U.S., and exported 313,617 head of feeder steers and spayed heifers (See Figures 1 and 2).

While these additions are a small portion of the total United States cattle inventory, the disease threat they pose is a significant issue. Chihuahua producers are able to realize the additional value of the U.S. cattle market, which can pay up to 30% more than their local market, after the cost ($40-60 per head) of preparation for exportation (Perez-Pria, 2007). There are economic benefits on both sides of the border, as Mexican cattle are known for their hardiness and ability to adapt quickly. A Texas producer notes:

> "Stress is a non-issue for Mexican cattle. Our naïve, native cattle have never had a stressful day in their life, but a Mexican calf has had stress every day of its life. So when they come over here, they think they are in heaven. And their consumption shows it"
> Jack Scoggins, Jr.

Referring to the pre-conditioning time needed to prepare Mexican cattle for a feedlot (7 days) compared to native cattle who adjust more slowly (21 days) and need extra time Rutherford, 2007).

The economic benefit of an animal identification/trace-back system is often in the resources NOT spent in reaction to an animal health crisis. The benefit lies in the ability to detect and limit the spread of a disease, enable faster trace-back of infected animals, limit production losses due to disease prevalence, reduce the cost of government control, intervention and eradication and ultimately minimize potential trade losses (Disney, 2001). While no dollar amount can be assigned to an outbreak of an animal disease, the effects of loss of trade due to an animal health threat are easily understood. Decisions by the United States and many other countries to cease trade with any country where tuberculosis (or many other diseases) is endemic demonstrates the impact of animal disease.

Bovine Tuberculosis. Bovine Tuberculosis is a disease of great concern for livestock producers. Not only does this disease have an affect on animal performance and health, but the resulting economic impacts are often significant. Bovine Tuberculosis (TB) is caused by the organism *Mycobacterium bovis* a Gram-positive acid-fast bacterium that is closely related to the organisms that cause avian tuberculosis, human TB and Johne's disease (OIE, 2005)

Tuberculosis is most often spread by healthy animals inhaling aerosols from infected animals. The infected animal can shed the bacterium in respiratory secretions, feces, milk, in some cases urine, vaginal secretions or semen. Cattle kept in production systems that comingle animals, constantly add new animals and have high stocking rates usually have a higher precedence of TB. Dairies and feedlots are known for having the highest incidence of TB, due to the constant influx of animals and high concentrations of animals in confined areas.

A TB eradication program began in 1917 that required all herds to be tested, and this program was highly successful in lowering the prevalence of bovine tuberculosis. During the 1950s, the inspection and surveillance program shifted from farm testing to abattoir surveillance. This methodology relies on meat inspectors to identify and collect samples from suspect animals, after sampling the samples must be sent to a laboratory for diagnostics. These inspections are conducted by two groups within the United States Department of Agriculture (USDA): Food Safety and Inspection Service (FSIS) and Animal and Plant Health Inspection Service (APHIS). In the slaughter plant, FSIS is responsible for ante-mortem and post-mortem inspections. If an animal is suspected to be unfit for human consumption, the FSIS has the ability to condemn the animal prior to slaughter. If the animal passes the ante-mortem inspection, it is then harvested and undergoes a second inspection after harvest (post-mortem). If the carcass is condemned after post-mortem inspection, the animal will not enter the human food-chain. Samples from the condemned animal are collected and sent to APHIS for further epidemiological investigations. After confirmations of a positive test, the APHIS investigators will begin an investigation to find the herd of origin.

Harvest reports from 1998-2002, that recorded the number of cattle harvested and the number condemned for tuberculosis (both from routine slaughter and reactor cattle sent to slaughter), show that less than one percent of animals at a harvest facility are condemned for having evidence of bovine tuberculosis. In 1998, of the 34.7 million cattle that were harvested, 17 were condemned for tuberculosis; in 1999, 34.9 million cattle were harvested of which 22 were condemned for TB; in 2000, 36.2 million head were harvested with only 7 condemned for suspicion of TB; in 2001, 38.9 million head were slaughter and of those 42 were condemned for suspicion of TB; and in 2002 32.4 million head were sent to harvest and of those 130 were condemned for evidence of bovine tuberculosis (Kaneene, 2005). With the low level of suspicion, it is very important that all suspected cases of bovine tuberculosis have samples submitted for diagnostics. In 2000, the goal was to have five submissions of suspicious lesions per 10,000 head of adult cattle killed at a given slaughter facility (Kaneene, 2005). To aid in meeting these goals, the bovine tuberculosis program began monetarily rewarding FSIS inspectors and APHIS veterinary medical officers, based on lesion submissions. If a submitted sample is positive by histopathology, $100 per steer or $500 for an adult animal is awarded, if the sample is culture or polymerase chain reaction (PCR) positive the award is doubled. If APHIS veterinary service investigation locates an infected herd a $6,000 award is shared between the FSIS inspectors and Veterinary medical officers on the case. This incentive program has significantly increased the number of submissions since its beginning.

In a telephone survey evaluating the successes and challenges facing the bovine tuberculosis eradication program in the United States, FSIS inspectors listed animal identification as one of the biggest challenges for the program. Inspectors reported that the large variety of identification methods being used and requirements varying between states is problematic. Additionally, the recording keep methods of harvest facilities varies greatly depending on the size of the plant – large plants are more likely to have formalized record keeping systems, while smaller plants are more likely to have simple paper-based systems. The information that the abattoir collects is dependent on the quality of information that is supplied by the feedlot, sale barn or any combination of sources. The inspectors also indicated that plants have difficulty keeping identification with all parts of the carcass (FSIS, 2002). Nearly one-third of FSIS inspectors surveyed also indicated that they were concerned that the system in place would not be sufficient for a major animal disease outbreak (FSIS, 2002).

Despite all of these efforts, TB is still a major animal health concern. To better handle these situations, the USDA designed an accreditation system that allows states to be classified independently or have split-state status to better benefit the livestock commerce in the area while still containing the area containing herds that pose a threat. There are five TB status levels (See Table 1), with three currently being used in the United States and the remaining two primarily used to classify Mexican states.

As of April 15, 2008 Michigan and New Mexico are listed as the only states with split classification. Michigan has three zones within the state – modified accredited (MA), modified accredited advanced (MAA) and accredited free (Free) New Mexico has a split-status with modified accredited advanced (MAA) and accredited free (Free) zones (Veterinary Services, 2008). These zones are usually defined by a geographical area, political, manmade or surveyed boundaries with mechanism of disease spread, epidemiological characteristics, and the ability to control the movement of animals across the boundaries of the zones taken into account (Beals, 2006a). These zoning regulations allow a small portion of a state to be sequestered and treated differently from the rest of the state. Before the USDA allowed these changes in zoning classifications, state boundaries were strictly enforced – so that a whole state was lowered in TB status, regardless of geographical boundaries or production differences.

Table 1. Bovine Tuberculosis Status levels

Status Level	Requirement to Advance to next higher level	States/Zones in this level
Accredited-free States or Zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis, and justify continuation of this level by annual reports and USDA reviews and evaluations*	*All in the U.S. except Texas, and zones in Michigan and New Mexico*
Modified Accredited Advanced State/Zone	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and have zero herd prevalence and [illegible] for 2 to 3 years after depopulation of last TB positive herd*	*Texas, Michigan, Minnesota and the New Mexico zone in the Clovis-Portales area*
Modified Accredited States or Zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and herd prevalence of less than 0.01% of cattle and bison in the state or zone*	*One zone in Michigan*
Accredited preparatory States or zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and have herd prevalence of less than 0.1% of cattle and bison in the state or zone*	*None in the U.S.* *Majority of Mexican States are at this level.*
Non-accredited States or Zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and have herd prevalence of less than 0.5% of cattle and bison in the state or zone*	*None in the U.S.* *Few remaining states in Mexico at this prevalence level.*

Table 1. **Bovine Tuberculosis Status Levels**		
Status Level	**Requirement to Advance to next higher level**	**States/Zones in this level**
Accredited-free States or Zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis, and justify continuation at this level by annual reports and onsite reviews and evaluations*	*All in the U.S. except Texas, and zones in Michigan and New Mexico*
Modified Accredited Advanced State/Zone	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and have zero herd prevalence and no cases for 2 to 3 years after depopulation of last TB-positive herd.*	*Texas, Michigan, Minnesota and the New Mexico zone in the Clovis-Portales area*
Modified Accredited States or Zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and herd prevalence of less than 0.01% of cattle and bison in the state or zone*	*One zone in Michigan*
Accredited preparatory States or zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and have herd prevalence of less than 0.1% of cattle and bison in the state or zone*	*None in the U.S. Majority of Mexican states are at this level.*
Non-accredited States or Zones	*Demonstrate authority and infrastructure to comply with the Uniform Methods and Rules for Tuberculosis and have herd prevalence of less than 0.5% of cattle and bison in the state or zone.*	*None in the U.S. Few remaining states in Mexico at this non-status level.*

Forms of Animal Identification

Brands. Brands are placed on the animal on a specific location (ex: hip, shoulder rib) and have a specific design to designate a particular owner. The majority of branding is done with a hot-iron brand that is heated and when the temperature is sufficient to provide a quick transfer of the brand to the hide and the iron is applied to the specific location on the animal. This type of branding destroys hair follicles that come in contact with the branding iron's design, leaving a bald pattern in the shape of the producer's brand. Freeze branding is another method of branding that requires more equipment to produce an identified animal. The branding iron destroys pigment producing cells within the hair follicle, leaving the desired design on the hide and subsequent re-growth. This method requires more inputs such as dry ice, alcohol or liquid nitrogen to maintain the branding iron's temperature. For best results the animal will need to be clipped and cleaned in the area intended for branding (Hall, 2004). While freeze branding is intended to not produce the bald patch of the hot iron brand, if the iron is left on too long, it will produce results similar to the hot iron brand. Conventionally, most branding is done to designate group or lot identification. It is a basic permanent form of identification for owner identification, but traditionally not individual identification.

Tattoos. Most cattle breed registries require registered animals to have a tattoo of their registration number in the ear of the animal. Some producers put the animal's permanent identifier as a tattoo, so that in the instance of a tag loss later in life, identity can be easily restored. Tattoos are fairly simple to apply, a tattoo gun comes with a set of characters and the gun is placed between the top vein of the ear and the second to the top vein, after the indentations have been made tattoo ink is rubbed into the indentations producing the identifier desired. (Gregory, 1996). The main concern with tattoos is that the identifier is not readily visible, often requiring an additional method of identification. Furthermore, it takes practice and skill to consistently produce quality, readable tattoos. The frequency of illegible tattoos is a point of concern, especially in the transfer of ownership. Tattoos are best suited to maintain the individual identity of a registered animal, but are not easily applied in large production settings as the only form of identification.

Ear tags. Ear tags are the most easily recognizable form of animal identification across species and production methods. They provide a relatively easy means of identification that is readable from a distance. They offer a variety of identification options – non-permanent, color coded, individual identifiers that are easily read and fit in most production practices. Tags are simple to attach to the animal, coming in a variety of forms. Metal tags are a one piece apparatus that is inserted into the ear, and clamped to secure. These tags are low cost, easily inserted and only require a specific clamping tool. They are used often for herd health programs (such as the Brucellosis vaccination program). Some plastic tags come in a male/female set that requires a tagging gun to attach both parts of the set to the animal. Other plastic tags require only a tag applicator to insert the one piece tag, these are also known as feedlot tags, as that is where they are most often used for lot identification while completing the finishing phases.

Radio Frequency Identification. Radio Frequency Identification (RFID) for cattle has gained credibility in recent years, and looks like it could be a major component of a national identification program in the United States. Radio Frequency Identification is used as part of an ear tags based system. Each RFID ear tag is comprised of a transponder which houses a microchip and a metal coil antenna. The microchip is responsible for handling the unique number for each tag, and the accompanying metal coil acts as an antenna which transmits information to the transceiver. The transponder is often embedded in a heavy plastic material that is shaped in the form of a button ear tag. The transponder communicates with a transceiver (or reader), which is usually a hand-held unit or stationary unit that is within close proximity to the animal (within 18 inches). The information collected by a transceiver is sent to a data accumulator – usually a computer, personal digital assistant (PDA) or other electronic device. The data that are accumulated is transferred to processing software that converts the information into a useable format for the producer (Michigan, 2007).

There are two types of transponder and transceiver communication. The first is a half-duplex relationship, which allows communication to be received only one way at a given time. Full-duplex communication allows the transponder and transceiver to communicate with each other simultaneously. As an example, a half-duplex relationship would be similar to talking on a walkie-talkie (e.g., one-way radio) that allows a person to only speak or listen at any time. A full-duplex communication is similar to a telephone conversation where the participant can speak and listen at the same time.

Signals for the transponder to the transceiver can pass through a variety of materials, but there can be interference with electric motors, fluorescent light and metal objects. However, for most practical purposes tags are read within a distance of 18 inches, where interference should not be a problem. A recent study looked at the readability of 13 RFID ear tags using three multi-panel readers for beef cattle, and found that once corals were modified for RFID technology (removing extra metal around the panel readers etc.) and using the latest technology produced read rates of 99% (Wallace et al., 2007). A key point of this trial is that reading success was a mixture of trial and error and modifications to accommodate technology. If commercial users are willing to adjust their system and standard procedures an RFID system can be used successfully.

Another concern with RFID technology has been performance under varying environmental conditions. It has been commonly discussed that there is a potential for the communication between transponder and transceiver to be affected by temperature. Wallace et al. (2006) found that as temperatures became colder (22°C, 2°C to -19°C) tag read range decreased slightly (0.07cm, *P*>0.05). Half-duplex tags consistently had the longest read range compared to full-duplex tags (*P*>0.05). The average read distance for readers varied between 8.6cm to 25.5cm, showing a significant difference between transceivers (*P*>0.05). The reading range of each transceiver inconsistently fluctuated as temperature decreased. However, despite differences in reading distance, all readers and tags worked under varying climates.

This research indicates that RFID technology can be used successful to identify animals. For producers to successfully use this technology dependent form of identification, they will need access to a computer and software to upload data. This system will likely find the highest success when coupled with a traditional ear tag to be used for ranch identification.

Injectable transponders. Implantable electronic transponders (IT) can offer a very secure and reliable method of individually identifying animals, and are relatively tamper resistant. Numerous studies have been conducted to determine the best transponder size, type and placement for the most effective animal identification. Santamarina et al. (2007) studied the abattoir performance of a variety of identification methods in Spanish pigs. Recovery of interperitoneally injected transponders was 89% from the viscera during time provided on the line between each animal, but was dependent on line throughput (abattoir A: 7 to 8 pigs/s compared to B: 6 to 6.5 pigs/s; $P < 0.01$). Of those transponders that were not recovered on the line, 9 were found in the bladder (0.8%), and 11% were reported as lost. However, Santamarina et al. (2007) reported that no transponders where found in the carcasses at the end of the slaughter line. In a corresponding study, Gosalvez et al. (2007) reported the on-farm finding for the same study, where animals where introperitoneally injected with glass encapsulated half-duplex or full-duplex transponders and where compared with the other methods of identification. Injectable transponders where recovered immediately after evisceration, when the gastrointestinal tract was removed for official veterinary inspection. If the transponder was not recovered immediately the viscera was removed from the line for later checking. On farm, transponder loss and malfunction were considered to be failures, there was no reported difference in failure rate between half-duplex and full-duplex technologies at the 240d readings (P>0.05). Gosalvez et al.(2007) reported that intrapertoneally injected transponders had a low failure rate (3.6%) but greater than the range provided from previous research conducted by Caja et al., 2005 and Babot et al., 2006 (0.4 to 2.0%; respectively). Gosalvez et al.(2007) reported transponders showed 100% readability between the farm and abattoir, with no negative impact on readability resulting from slaughter practices in Spanish pigs.

In cattle, a study was conduced by Conill et al. (2000) to determine the ideal location for injectable half-duplex transponders to be placed, based on size (23 or 32 mm) between three locations: armpit, ear scutulum (cartilaginous portion of the ear) and upper lip. Transponders were injected by both trained and untrained operators in 1 to 3 mo calves (Conill et al., 2000). Losses were compared by location, and the 32mm located in the lip had the highest level of loss (P<0.05). Conill et al. (2000) reported an increased time of (51 ± 4 s; P<0.05) for insertion of the transponder in the lip, (37± 3 s) for those injected in the armpit and (44 ± 3 s) for those placed in the ear; the increased times for insertion in the lip and ear was expected as the animal's head had to be completely immobilized for proper insertion. There appeared to be no effect of injectable transponders on the animal's performance, however, there was a low percentage of animals injected in the lip (5, 3.3%) and ear (4; 2.1%) that formed small infections with the formation of an abscess and rejection (loss from the body) of the transponder. These results, while low, raise concerns on an animal stewardship front, as these occurred under experimental conditions, and infection would likely increase under typical production settings. Additionally there was a reported increase in animal reaction to the transponder insertion with those placed in the lip, compared to other locations. Conill et al. (2000) reported that readability of the transponders decreased over time with ear and lip locations (*P*<0.05), while 84.0% of those losses were recorded by week 3. This would indicate that with increased training and sanitation during injection that losses could be reduced. Average losses of transponders were greater in the lip 14.0% (*P*<0.05), than in the ear (5.2%) and in the armpit (1.7%). Overall transponder performance was reported better in transponders located in the armpit, due to reduced losses and the capability of the location to accommodate both sizes of transponders. Transponders located in the lip had the highest level of retrieval at the abattoir at 99.2% compared with 96.7% and 96.7% the armpit and ear, respectively. These results are likely due to the lip not being a cutting location on the abattoir line. Mean recovery time for the lip was the quickest (27 ± 2s), then the ear (52 ± 5s) and lastly the armpit (75 ± 7s). These results indicate that a slow chain speed (similar to those in Spain, where the study was conducted) would be needed to allow transponder recovery if animals were identified by injectable transponders.

In similar studies, Fallon et al. (2002) studied the effect of five injection sites using five varieties of transponders, differing in weight, length and the covering material (glass or plastic). Of the five sites, there was poor transponder performance from two sites in the ear, as the transponders broke as a result of impact after the animal was stunned, sites D (4.5 cm needle inserted subcutaneously to its full length towards the base of the ear, but downwards at 45 degrees to its long axis) and S (middle of the caudal surface of the ear, 4cm from its base) (Fallon et al., 2002). At the abattoir, post slaughter readability was 88.2% and 68.1% readability, respectively. These results suggest that a better location for transponder placement is needed for effective use of injectable transponders. Further research was conducted (Fallon et al., 2002) to determine the effectiveness of IT placed underneath the cartilage of the ear, offering protection to the IT from the cartilaginous portion of the ear; injection site two was at the caudal base of the ear, at a right angle to its long axis. Concerning recovery, it was found that 8% of the transponders migrated more than 5cm from injection site two. The injection site that was underneath the cartilage of the ear allowed for much better recovery in the abattoir, however, it showed a 10% failure rate in bulls, which would suggest that the aggressive behavior of bulls is not conducive for IT placement in the ear.

A third study (n=30) (Fallon et al., 2002) was conducted to determine the best location between placement in the ear underneath the cartilage or a location in the upper lip in bulls. This experiment provided 100% readability from d0 to the abattoir post-slaughter. However, post slaughter recovery of the IT proved to be a problem, as 45% of the large ITs (28 mm long, 3.6mm diameter) remained in the head, and it was necessary to remove the head from the line for further dissection to locate the IT. The smaller (19 mm long, 2.8mm diameter) ITs had a 11% non-recovery rate. Through their extensive studies, Fallon et al (2002), noted the difference in injection devices and the performance of the ITs based on injection method. They suggest that placement in the lip is not suitable on "aesthetic and animal welfare grounds", and was difficult to recover the IT post-slaughter. The recommended location would be placement underneath the cartilage in the ear (Fallon et al.; 2002). Both studies suggest that implant location and implantation technique have large impacts on readability of the IT. In the US, consideration should be given to chain speed at the abattoir, where such long recovery times seem unreasonable under current fabrication systems. The potential for ITs to be damaged would increase with the use of growth implants, which are placed in the ear, often in the same general area, the implant gun could have detrimental effects on the performance and readability of the IT. IT migration is a potential food safety and product quality concern that will need to be addressed with advanced recovery methods in the future.

Boluses. Boluses are used in ruminant animals, traditionally as a means of delivering a slow release substance (trace minerals, anthilmentics etc.) to the animal. When properly inserted they reside in the reticulum (second stomach) of the animal and are intended to remain with the animal until the capsule dissolves or when the permanent bolus is removed at the abattoir. A ruminal bolus is inserted one of two ways: using an esophageal balling gun which delivers the bolus to the top of the gullet or by hand. Digital boluses intended for animal identification can be made from a variety of materials including ceramic, heavy weight plastic, steel or even glass depending on the manufacturer (Fallon, 2002). These boluses have the potential to include GPS components, thermometers to measure internal body temperature as well as transponders for identification. These components can be inserted into a cavity in the bolus and the cavity is sealed with an epoxy resin. Proponents of the ruminal bolus believe that they are secure, not easily tampered with, have a high retention rate when placed properly and can be reusable. Fallon, Rogers and Early (2002) stated that electronic rumen boluses have the advantage over an injectable implant, as they avoid potential contamination of meat or by-products, eliminating food safety concerns. Inserting a foreign object into the rumen could raise concern over animal welfare and performance. No differences in average daily gain and dry matter intakes were found between bloused and control (no-bolus) lambs (Ghirardi et al., 2007). Specific density of the bolus is a critical component of bolus retention, as the bolus has to be denser than the contents of the rumen. Riner et al. (1981) reported that a specific density of $1.6 g/cm^3$ was required to prevent regurgitation from the rumino-reticulum, and a minimum of $2.0 g/cm^3$ for reticulum retention. Boluses with a specific density of 2.45 to 2.75 g/cm^3 showed

100% retention (Fallon, 2002). Fallon speculates that boluses need to have a specific density more than twice that of rumen fluid in order to avoid loss through regurgitation. Loss through regurgitation has been shown to be impacted by diet content. Diets high in coarse hay produce a rumen that has a large, dense floating layer beneath a gas dome, with the liquid components and suspended fiber beneath. The floating portion is comprised of the most recently ingested forages (Fallon, 2002). This layer would be regurgitated to allow the animal to chew its cud, and should the bolus be sitting on this layer, it too would reoccur in the animal's mouth, and would be "tongued out" by the animal. Fallon (2002) also shows a four-fold increase in bolus loss in adult cows verses growing cattle, attributed to the higher roughage content of the adult animal's diet, and subsequently thicker top layer within the rumen. Finding an identification method that is low stress and easy on the animal is very important to the ready adoption of any new method of identification. Two lambs in the studies conducted by Ghirardi et al. (2007), showed profuse salivation, nasal discharge, panting and general depression after administration of the bolus. The boluses were palpable in the neck and x-ray showed the bolus lodged in the esophagus. While the bolus was dislodged using a piece of plastic tubing to gently push toward the rumen, it proves the point that a trained and skilled operator is very important for success of the device; not only learning to properly use the insertion equipment but recognizing the importance of proper restraint and animal positioning in bolus application.

Ruminal boluses can be read by two basic variety of readers, a stationary panel-type (used in areas with a high volume of cattle movement – typically an auction market, etc.) or a hand-held reader used in smaller production settings. Given that a bolus with the correct specific density has been chosen, retention rates can be upwards of 97% (Fallon, 2002). Retention tends to remain constant if animals can retain the bolus through the first hours after application; however, when the bolus was lost another was inserted at a later date and retained

(Caja et al., 1999). When choosing a time to insert the bolus, body weight seems to be the preferred indicator insertion over a specific age in lambs (Ghirardi et al.,2007). These boluses are often expensive, and therefore cost prohibitive for many producers. They companies that produce these boluses market them as a re-usable product that can be recovered in the abattoir and re-assigned to another animal which allows the producer to spread the cost associated with the technology over more animals. Fallon, Rogers and Earley (2002) describe the recovery of the ruminal bolus to be relatively easy; they mention that additional steps may need to be added to keep the rumen from turning over during its movement from the abattoir line to the offal area. However, it should be noted that this suggested modification is for 'fast moving slaughter lines - 100/head per hour'. Obviously, this would be a much larger concern in the United States where the chain speed is likely to be four-times that. Caja et al. reported that recovery time varied between 12 to 15 seconds per animal for cattle at an abattoir in Spain, which again could raise concern for the faster U.S. chain speed. While ruminal boluses have the advantage over an implanted microchip in avoiding potential food safety problems, the practical use of the rumen bolus is still in question.

DNA testing. DNA testing uses the ability to determine SNPs (single nucleotide polymorphisms), which are variations in a DNA sequence. SNPs are used to measure the genetic variation within species, and can be used to identify individual animals, under the assumption that samples were collected correctly and archived for future reference. In a study conducted in Nevada, DNA testing was done to improve bull culling decisions (Gomez-Raya et al.,2008). This study did little for individual animal identification, but was able to prove parentage based on 12-15 specific markers. MMI Genomics, Inc., IdentiGEN and similar companies have advertised their technology's ability to establish identity in a manner that is "unique, permanent and tamperproof" (Holm, 2005). This DNA-based technology has the potential to aid with breeding decisions, animal management and allow certification of branded products. IdentiGEN has had some success in Europe. The company claims to have "DNA Identity Control" through the DNA Traceback® which indicates they can maintain identity of a carcass through the supply chain (IdentiGEN, Products and Services).

This trace-back system requires participation from all sectors of the supply chain, with continuous sampling from live animals/carcasses and from retail outlets. This technology no doubt holds a potential to determine animal identification, parentage, breed characteristics and more. However, the specifics requiring continuous sampling and archiving would prove to be problematic for many animal production sectors. Perhaps the biggest disadvantage would be cost. Each sampling and archiving activity would accrue some cost, and spreading that cost through the production chain would be important. The labor required to sample would be substantial on all accounts, but the additional resources needed to archive, transport and maintain the samples could be cost-prohibitive. They describe their process as a system where abattoir employees are taught to collect samples from every bovine or porcine animal that goes through the facility. The sample is then sent to IdentiGEN® for DNA profiling, which usually takes less than 48 hours (Charlton, 2008). This again is a situation where the chain-speed of the U.S. system must be compared to countries where this program has had success. In the European Union, the rate at which the abattoir can process animals is likely to be 1/3 of U.S. capacity.

Additionally, this DNA-based technology does little for real-time animal identification, as samples have to be sent off for analysis before any conclusions as to identity could be made. Relying on DNA-based identification seems unrealistic as producers, feeders and processors would still have a need for a visual identifier.

The DNA-based identification seems to be more successful in applications that see the benefit of genotyping their products for value added programs. The Nevada study indicated that genotyping 15 microsatellites with 20 calves per sire resulted in benefits of $1.71 to $2.44 per dollar invested at bull culling rates of 0.20 and 0.30 (Gomez-Raya et al., 2008). How that value would cross over on a per carcass basis is yet to be seen. IdentiGEN® promotes the ability to determine if products are natural, organic or meet breed specifications for particular marketing options.

To project that DNA-technology is the future of individual animal identification would be pure speculation, however, it is certain that DNA-technology will influence marketing and trading. While other methods of identification are potentially more economically, a method that can not be tampered with, that stays with the animal will prove to be most valuable.

Retinal Imaging. Retinal imaging uses the unique pattern of the retinal vasculature pattern in the eye to establish individual identification. The highly vascularized retina of the ruminant, pig, dog and cat is characterized by the presence of large vascular network in the light-sensitive portion of the retina. The blood vessels extend from the optic disc to the jagged margin between the light-sensitive and light-insensitive portions (De Schaepdrijver et al., 1989). The branching of the blood vessels is what makes each eye unique. The vessels branch off from the central retinal artery in what is known as Laplacian growth, which is found in nature (Peterson, 2001). Laplacian growth is seen in nature by how rivers diverge, how glass breaks and how trees branch. This growth is influenced by many random factors. The retinal blood vessels develop during the second half of gestation (Huntzinger and Christian, 1978). In a study conducted using identical twins, measurements were taken to quantify the retinal vasculature pattern of identical twins. Comparisons were made between identical twins and between each individual's eyes. This study showed that each retinal vascular pattern was different and unique even between identical twins, which had the same gestational environment (Huntzinger and Christian, 1978).

The Optibrand Technology is a system designed to photograph the retinal vascular pattern (RVP), using a digital video camera that transmits full motion video at the rate of 19 frames per second. This camera can be used to capture the retinal image or any other item (e.g. ear tag, brand etc.) that would be stored with that animal's records. This camera is connected to handheld computer (OptiReader®) with a rechargeable battery. The controller (handheld computer) has a GPS receiver within it. When the OptiReader® is in use, the latitude and longitude, time and date are automatically encrypted on each animal's record. The OptiReader® has the capability to read RFIDs, barcodes and has the capability to allow users to custom design data fields to be collected. The records are stored on a removable CompactFlash card, and imported to a personal computer for storage. The OptiReader® is accompanied by Data Management Software (DMS) that allows collection and management of records.

To test the effectiveness of using retinal imaging to permanently identify animals, 491 beef and 220 sheep 4-H projects were retinal imaged at the time of project enrollment and compared to traditional project identification of nose printing. Rusk et al. (2006) reported that retinal image collection for beef animals and sheep took longer than nose print collection (38.98s, 56.03s vs. 25.25s, 22.25s; respectively by species; $P<0.05$) and collecting retinal images from sheep took significantly longer than collecting images from beef animals (56.03s vs. 38.98s; $P<0.05$). Reading and verifying nose prints is a skill that requires extended amounts of practice, and can still be misleading. To further compare the two technologies, the researchers asked 38 adult volunteers to determine the visual verification of retinal images and nose prints for both beef and sheep. The volunteers were given 20 pairs of retinal images (10 from beef and 10 from sheep) and 20 pairs of nose prints (10 from beef and 10 from sheep) and asked to determine if each pair was a match by circling YES, NO, or UNSURE. (Rusk et al., 2006). The volunteers were given a short description of each method of identification, but were considered untrained volunteers. The volunteers were able to correctly match 68.94% of the nose prints, and 8.42% reported being unsure of the correct match. Concerning beef animals and retinal imaging, 98.64% were correctly matched and only 0.27% of answers were recorded as unsure. With sheep, nose prints were accurately read for 79.47% of sheep nose prints, with 5.26% being marked as unsure. For sheep retinal images, 84.86% were correctly matched; none were reported as unsure matches. Rusk et al. (2006) reported a 29.7% advantage for correctly identifying retinal images from beef cattle over nose prints, there was a trend for this to be true for sheep as well. With matching, there is a potential for false-matches (identifying a pair of images as a match, when it is in fact not a match) and false non-matches (indicating a pair of images or nose prints were not matches, when in fact they were a match); Rusk et al.(2006) reported that false match and false non-match rates of visual verification of retinal images were lower than the rates for nose prints. There was a trend for participants to have higher scores matching beef images than they did on sheep images; although there are no structural differences in the retinal vascular pattern of beef and sheep.

A Northern Ireland study conducted by Allen et al. (2007) evaluated the use of the Optibrand System and the accompanying data suite as a method for identifying cattle. Eight hundred sixty nine head of animals were retinal imaged, creating an initial enrollment of 1,738 retinal images (2 eyes per animal). An additional 2,266 retinal images were collected to be used for verification at different stages of production. For their analysis, all retinal images were visually compared prior to initiating computational comparisons utilizing the DMS. Allen et al.

(2007) reported a 98.3% (2,227/2,266) success rate for computationally matching the initial enrollment image with an image collected at a later date. The remaining 1.7% (39 retinal images) were matched using visual inspection. (Allen et al., 2007). Combining visual and computational matching allowed 100% matching for this particular study. While these rates are feasible, consideration should be given to the fact that each image pair was visually inspected prior to computational matching. This would not be a realistic situation for most production settings, the time required to double-check each image prior to matching, essentially negates the purpose of the DMS software. While this method did allow the study a high level of success, the same procedures could not be expected in a commercial setting.

In addition to the above verification study, Allen et al. (2007) completed an ear tag alteration simulation in which ear tag information was switched between retinal images after the initial enrollment and subsequent imaging sessions. This allowed the Optibrand System to be tested in a real-world scenario when human error or fraud would cause switching of animal identification. Of the 115 animals involved in the simulation, 100% resulted in non-matches when comparing the initial enrollment image with an identification-switched later image (Allen et al., 2008). The non-matches were confirmed by visual inspection. This is a strong example of the power this technology yields in the case of mistaken or misleading identities. If the operator has the dates of when the animal was last scanned, making the comparisons would be fairly simple, and the answer could take minutes to achieve with a reliable internet connection, personal computer and knowledge of the software. The most obvious benefit is that this type of verification could be done on site, without the need to send samples off for lab analysis, and can be successfully completed with minimal training. The degree to which the animals can be successfully identified in a repeatable, verifiable manner is highly dependent on the amount of training that the operator has received. These are considerations that must be taken into account when deciding on a successful identification method.

Case Study: Other Identification Systems

New businesses often model their business structure after another longer established, successful business. This allows the younger business to take the hard-earned successes from the model and manipulate the details to fit their needs. The formation of a national animal identification program would be similar in that studying successful systems could help the United States develop a successful system of its own. The best countries to look at are perhaps the ones that export a majority of their products (e.g., Australia and Canada), because importation regulations for many countries are typically more stringent than a country's policies for its own producers.

Australia. Australia livestock production is primarily export-driven. In 2006-07 Australia exported 67.1% of its total beef production (MLA, 2007). The large proportion of beef cattle exported indicates that a larger percentage of beef producers are familiar with the exportation process, and therefore rely on exportation for business/operation success. Australia has used a tail tag system over the last 30 years to identify the most recent property of origin for cattle. The tags cost producers about 2 cents each, and will remain with the animal approximately 30 days. The tail tags are required to be applied to each animal before each transaction. This tag links the particular lot or pen of cattle with a Property Identification Code (PIC). In 1996, following an issue with pesticide residues, a National Vendor Declaration and Waybill (NVD) was created. This paper-based system includes assurance by the cattle owner whether the cattle have been treated with hormonal growth promotants, produced at a location that that uses practices consistent with an independently audited quality assurance program, born and raised on the vendor's property, if they had been fed any by-product feeds in the last

60 days, are still within a holding period for treatment of any drug or chemical or had grazed or been fed fodder at risk for endosulfan spray drift. The NVD is completed by the seller before a transaction occurs. This form is not mandated by the Australian government, but is demanded commercially. A NVD is required for all animals intended for exportation. In addition, this document is considered to be legally binding, and can be used for liability in the event of a legal claim by future owners of the cattle or beef for which the NVD was completed (Tonsor and Schroeder, 2007).

In addition to the NVD, there is now a National Livestock Identification System (NLIS). The NLIS is a whole-of-life identification system that allows individual animals to be traced from birth property to slaughter destination. The NLIS requires that all calves have Radio Frequency Identification (RFID) applied before the animal leaves the place it was born. Readings are mandatory at each cattle transaction; with each movement a history of the animal's life is compiled. All information is kept on a centralized database, maintained by Meat and Livestock Australia (MLA), an industry-funded service organization funded by levies obtained from livestock producers from each animal transaction. This program also gives producers the opportunity to use the system for both keeping track of animal movements and managing individual animal production records as well. If a producer is so inclined, they can keep track of medical records, growth performance, pasture performance, purchase and sale dates and carcass data (Tonsor and Schroeder, 2007).

In 2007, the Australian Department of Agriculture, Fisheries and Forestry (DAFF) conducted a trial called "CowCatcher 2" to evaluate the effectiveness of the NLIS. Three hundred tag numbers were randomly selected from a variety of industry segments (cow-calf, feeders, sale barns, etc.) within the production chain; over all regions of Australia so that all jurisdictions were adequately represented based on a 12 month average of transactions. In this trial, 98.7% of all cattle were traced back to their property of birth within 24 hours (NLIS, 2007). One state reported that the 57 head of cattle they were allocated to trace had the potential to contact 460,000 other head over their lifetime (DAFF, 2007). While this exercise is considered successful by all accounts, the participants in the study still had recommendations for improving the system. These recommendations include the ability to trace property-to-property movements, improve the epidemiological tools that would help in the event of an animal disease emergency and increase the size of the database when there is high usage (such as an animal disease emergency) among others (DAFF, 2007).

Canada. The Canadian Cattle Identification Agency began in 2002 and was designed to be a comprehensive identification system that would allow unique animal identification to all animals leaving their premise of origin. The Canadian Cattle Identification Agency (CCIA) is a non-profit industry led organization. The CCIA is led by representatives from all segments of the Canadian cattle industry.

The CIAA identification system is a tag-based system that allows each animal to be assigned a unique identifier that will follow the animal from birth-place to the abattoir. In 2003, the CIAA went to a RFID tag-based system that allows them to keep an "efficient and effective trace-back system" (CCIA, 2008). In order for a tag to be CIAA approved, the tag must have high retention rates and good readability among other characteristics. In addition, each tag has a CCIA identification number unique to the individual animal (including 124 which is the Canadian Country code), CCIA trademark (3/4 Maple Leaf and "CA" letters), be yellow in color with a yellow backing, and meet all requirements of the Canadian National Standards for RFID technology (CCIA, 2008). Official CCIA tags are available from 1,500 retailers (veterinary offices and feed stores) across Canada. The CCIA provides software for the retailers to report sales of CCIA tags. When a producer purchases the tags, the producer enters the premises identification number (PIN). The PIN number is linked to the producer's information – name, address, phone number, fax number and e-mail address. It should be noted that there is no information about the producer's animals or herd. At the time of purchase the retailer scans the tags in packages of 25, and they are then linked with that producer's information. Before an animal leaves a production site the animal must have a CCIA tag in place that will go with the animal as it goes through the production chain. A unique and helpful addition to the Canadian system is that the tags are retired at each ending point (packers, rendering wagons, etc.), so each tag would ideally have a beginning and an ending.

Prior to May, 2003 the CCIA conducted over 100 trace-backs for animal health purposes and all were reported to work in less than three minutes. The CCIA system was put to test during the BSE investigation of 2003 to find offspring from an infected herd. While the infected animal was too old to have been identified under the CFIA system, the system at the time would only have tracked the animal's movements if it was not intended for slaughter or exportation (Lawrence et al., 2003). The system that had caused such producer level discontent quickly became an unlikely source of optimism for the Canadian cattle industry when the CCIA identification system helped expedite the re-opening of foreign markets to Canadian beef (Murphy, et al., 2008).

Chihuahua State, Mexico. The animal identification system within the Mexican state of Chihuahua is export oriented and very successful. The Union Ganadera Regional de Chihuahua headquartered in Chihuahua City, Chihuahua, Mexico is the leading force for the development and implementation of the identification system. The Chihuahua system was developed in

2002, approximately the same time that the United States began developing its animal identification plans. In December 2007, a group comprised of USDA, Optibrand, New Mexico Livestock Board and Colorado State University researchers visited with animal health officials in Chihuahua City, Chihuahua, Mexico. The following is a report of the visit and the identification system in place within the state of Chihuahua.

The Union Ganadera Regional de Chihuahua is a very influential group within the state of Chihuahua, particularly among cattle producers. The Union was established in 1936 under the influence of visionary Chihuahua cattle producers. The goal of the Union is to meet the needs of producers and safeguard the interests of farmers in the state, for the benefit of society (UGRCH, B). The UGRCH is the only entity in the state of Chihuahua that is cleared to export cattle (UGRCH, A). The producer must work with the UGRCH in order to prepare their cattle for exportation. In order for a producer to have the ability to market their cattle within the state of Chihuahua they must be a member of the Union. The most unique and advantageous component of the Chihuahua system, is the regionalization of the state based on bovine tuberculosis status (see Figure 3). Areas with a large number of dairy cattle are classified differently from the rest of the state, as dairy animals tend to have a higher prevalence of tuberculosis. Areas are defined as Region A (Accredited Preparatory), Regions B1, B2, and B3 (Non-accredited zones).

Chihuahua Identification system. The Chihuahua identification system is based on the producers union and the governmental agencies working together. The Union office of Exportation is the main communicator between the regulatory bodies and the producer. In each county (municipo) there is a UGRCH office. When a producer is ready to move cattle off the site of birth, the producer contacts the municipo office and buys green metal Chihuahua state ear tags. These tags are allotted to producers based on the number of cattle the producer is known to have (e.g., if a producer is requesting 300 tags, and only has 100 head on record with the UGRCH, then a UGRCH employee would evaluate the production site to determine the need for additional tags). If the producer qualifies, tags are assigned to the producer through a computer database that is linked to Chihuahua City. This database links the owner's information – name, municipo and brand registration number. The green tag is always placed on the animal by the original owner, and will remain with the animal whether it is moved by the original owner, sold, or traded to a second owner.

In order for an animal to move from its birthplace, the green ear tag must be intact, but the producer must have also received a cattle movement permit (*pase de Ganado*). The cattle movement permit is required for any animal movements. To receive the cattle movement permit, proof of ownership or legal interest must be supplied. The permit is valid for only 10 days, for only one movement to the destination location on the trip permit; each permit is uniquely numbered. The permit includes the following information: person issuing permit, date of issue, name and address of permit owner, origin of animals as well as the purpose of the movement (e.g., exportation), the person responsible for cattle during movement, and intended destination. The cattle movement permit also contains information regarding who prepared the permit, how the animals will be moved (truck, trailer, etc.), and the test and certificates: *Certificado Zoosanitarion* (Mexican federal animal health certificate), *Certificado Brucelosis*, *Certificado Tuberculosis* indicating testing for both diseases, *Certificado A Garrapata* indicating tick testing and potentially a *Guia de Transito* when cattle have moved to another state within the Mexican territory. The person who has requested the *paso de Ganado* is included on the permit, the brand license number and the type of animal being transported (steer calves, heifers, etc.). If the cattle are traded with official documentation (invoice from seller) the invoice number is recorded, and the brands are drawn, and brand registration number recorded as well as the number of animals the invoice from the seller would verify.

There are approximately 21 casetas (check stations) throughout the state of Chihuahua located mainly on the major routes within the state, usually close to junctions with other heavily traveled routes (Figure 4)

These casetas (check stations) check all agricultural commodities – not just livestock. At the first caseta, the caseta personnel check that the trailers contains the animals the movement permit allocates and the trailers are sealed with an official seal. At each subsequent caseta the producer travels through on the way to their intended location, the caseta personnel stamp and verify the trailer and cattle movement permit. The casetas are not regulatory bodies, but if a problem does arise, the producer must turn around and clear the problem with the Union or regulatory bodies before travel can continue. The casetas are open 24 hours per day, and they keep track of the number and type of animals that are in movement. These records are kept on an Excel-based program, and are uploaded to the Chihuahua City every 10 days. The records include the intention of the movement (to slaughter, for export, etc.), where they are coming from, the owner and the cattle movement permits number. A major function of these check points is to keep cattle from tuberculosis rated 'B regions' from entering an 'A region'. Cattle from an 'A region' can not be offloaded in a 'B region' if they are intended for further movement or exportation. Cattle originating from 'B regions' cannot enter an 'A region' unless they go immediately to slaughter. Animals originating in a 'B region' must stay in a 'B region' until they are ready to be harvested (Carmona, TB 43) (See Figure 3).

The boundaries between regions often change due to depopulation efforts in the 'B regions'; typically the lesser status regions are predominately populated with dairies. The majority of Mexican states are zoned as Accredited Preparatory States or zones (analogous to a 'Region A'), while the majority with a higher incidence of tuberculosis are zoned as Non-accredited States or zones (analogous to 'Region B') (Beals, A). Cattle from non-accredited zones can not be export into the United States.

If an animal is intended for exportation, it will often go to a 'processing'/ holding pen on the outskirts of Chihuahua City to wait further processing and receive the final approval for exportation. These holding pens are highly coordinated and are maintained in specially regionalized areas of the outlying area surround Chihuahua City (residing in 'A regions', allowing cattle to be exported). In order for cattle to be exported to the United States, each animal must test negative for tuberculosis. The tuberculin test is mandated by the Mexican *Subsecretaria de Agricultura y Ganaderia, Pesca y Alimentatcion* (SAGARPA) – *Comision nacional de Sanidad Agropecuaria – Direccion General de Salud Animal.* The equivalent in the United States would be United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) – Veterinary Services (VS). Tuberculosis testing is done by a veterinarian employed by the holding pens or ranch and supervised by a veterinarian from *Comision nacional de Sanidad Agropecuaria.* The tuberculosis test is an injection of tuberculin in two folds of skin underneath the base of the animal's tail. Tuberculin is a bovine tuberculosis protein that will induce an immunological response in the animal if the animal has been exposed to tuberculosis. An animal that displays a reaction to the tuberculin shot does not always have an active case of tuberculosis, but has likely been exposed to the bacteria in its lifetime. These animals are termed 'reactors'. The tests are read (examined) after 72 ± 6 hours to check for any reactions. At this location, all cattle that are eligible for export to the US are branded with a '08' which is the state code for Chihuahua and are branded with an 'M' for Mexican steers or 'Mx' for

Mexican spayed heifers. Currently, the individual state brand requirements are set to cease by the end of 2008.

Female cattle that are intended for show, breeding or exportation are given a brucellosis test. This test uses a blood sample and is mixed with an antigen, if agglutination exists on the sample, then brucella is indicated. This is not a large concern for the United States, as currently all females crossing into the U.S. from Mexico must be spayed.

The paperwork that confirms the animals have passed a tuberculosis (and brucellosis test if needed) is sponsored by the *Campana Nacional Contra la Tuberculosis Bovina y Brucelosis,* which is the National Campaign against Bovine Tuberculosis and Brucellosis. The form includes information on the *dictamen*, an official letter written by the authorized veterinarian once the tuberculosis test is reviewed. Each form has a unique number. The cattle owner's name and address, the ranch name and the location the tests were conducted, reason for conducting test: exportation, movement to another municipo, or different state. The type/use of the animal is also indicated, whether the animals are used for milk, meat or both. Any additional identification the animal would have is also indicated – ear tags, tattoo, etc. The results from testing for tuberculosis and brucellosis is also recorded, the number of animals reacting positively, the total testing negative, the total number tested and the total number of animals in the herd (if tested on the ranch). The date and hour of when the tuberculin test was administered is recorded, as well as the dosage level and lot number of the tuberculin. The expiration date for the tuberculin is also recorded. The date and hour of 'reading' the test is recorded as well. There is space for the conducting veterinarian to make notes about the injection site before the injection and at the time of reading (72 ± 6h after injection). At the time of reading, the results are indicated as 'N' for negative or 'R' for reactor. The registration number, name and signature of the accredited veterinarian are recorded. The form also has space to record identification numbers for each animal, a short description of each animals breed, age and sex. While this form is a legal document, it does not allow for animal transportation, and the document expires 24 months after the tuberculosis tests are read. There is also space for animals that have lost ear tags and have been re-tagged, born into the herd, or added through purchase to be recorded.

While the animal is undergoing testing, an additional annex form is used to describe the origin of all animals within the lot. On the annex form the examining veterinarian fills out information required for each animal and puts a blue ear tag (official U.S. import tag) on each animal to be exported. On the annex form, a sequential list of all blue export tags for the lot is recorded, as well as the municipo of origin, the municipo's status, and the owner's name, farm of origin, the green ear tag, a brand drawing, and a reference to the tuberculosis test chart number. This form is signed by an examining veterinarian, as well as the federal animal health official overseeing the procedures. Once both have signed the paperwork, the official seal of the Mexican Animal Health in placed on the paperwork. This system is designed to allow multiple checks on the paperwork to ensure correctness. This built in redundancy of the 2 tag system and multiple checks o the paperwork allows identification to be reestablished in case of one of the ear tags is lost and helps prevent data recording errors.

At this time, producers have a certificate of movement, and have completed tuberculosis and brucellosis testing. The next step is for the owner to obtain a certificate of origin. The certificate can only be supplied by veterinary personnel certified by the federal government. The owner goes to the Chihuahua Bovine Tuberculosis and Brucellosis Eradication Sub-Committee. Two to three days before the cattle are set to be exported, certified veterinarians check records for each animal. Animal by animal the veterinarians check tuberculosis tests, green ear tags, and blue ear tags. The certificates are all uniquely numbered – a Chihuahua state code, consecutive number and the year. With this certificate the veterinarian certifies that all information on the certificate is true and identifies the herd of origin of the animals described. The exporter's name, signature, address – including town and county are recorded.

The second portion of this certificate for lot of cattle, records the number of spayed and castrated cattle. The sequential numbers of the blue export tags are listed, and with the number of herds the lot originated from. The herd of origin information is needed so that the veterinarians can certify that the cattle originated in herds that are authorized to export to the United States. The state or region that the cattle are coming from is listed, and the tuberculosis status is listed using the USDA classification scheme. After this information, the veterinarian must state that Holstein animals or Holstein crossbred animals are not included in the lot. At this time any annex (recording blue ear tags) forms that have been completed for cattle within the lot are attached to Certificate of Origin.

The final requirement is the *Zoosanitary Certificate*. The *Zoosanitary Certificate* is required for many agricultural commodities, not just cattle. It is especially important in moving animals between states where the requirements for movement are different. This certificate includes a unique certificate number, the purpose for the certificate (animal movement, movement of other agricultural products). The name and address of the person requesting the zoosanitary certificate are recorded, with information about the business or farm of origin, the location (town, city or village), municipo (county) and the Mexican state. Similar information is collected for the intended destination of the products/animals – including business name and geographical information. For goods/animals intended for export, the final destination is Mexican export facility at the port-of-entry at the U.S. border.

Next, information about the cattle or products being moved is recorded. The form will show the total number of animals in the lot, the species being moved (cattle, horses, sheep, etc.), the reason for transporting the animals is recorded (slaughter, export, breeding, etc.), the method of animal marking or identification is recorded (ear tag, brand, ear notches, etc.). If the load contains animal product, the specific product must be indicated (meat, lard, hides, etc.), and the purpose of the movement of the animal products (processing, consumption, etc.) and the packaging of the products and units of measurement are recorded.

For animal movement, this form must have the consecutive (blue) ear tag numbers listed, if different lots are transported, there is space to list consecutive numbers for each lot. The method of transportation being used is recorded (sea, air, overland, etc.). The make of the transporting vehicle, the license plate numbers. The verification points along the route where the cargo is inspected is also recorded. The tuberculin test numbers for exportation cattle are also recorded, as well as the sex of animals for export. The inspecting veterinarian also verifies that the cattle came from a tick-free zone. If the animals do not come from a tick-free zone, the treatment the cattle received and the federally approved veterinarian responsible for the treatment is recorded. Finally, if all of the above is correct and in the correct order, the UGRCH and the approved veterinarian certify that animals or products are not a zoosanitary risk and that all requirements for movement have been met. The location where the zoosanitary certificate was issued is recorded the date of the zoosanitary certificate was issued is and the date the certificate will expire.

Certificate of Ovariectomy. The certificate stating that heifers have been ovariectomized (spayed) is required for all female export animals intended for the United States. This certificate is issued by the USDA, Animal and Plant Health Inspection Service and Veterinary Services Western Region (USDA, APHIS and VS). A Mexican veterinarian that has been accredited by the USDA in Mexico is responsible for spaying the animals. When the ovariectomy is being conducted an official accredited Mexican representative will verify that the work is done correctly. The certificate of ovariectomy shows the name of the herd owner; with the location where the spaying took place. A note is written by the accredited Mexican veterinarian that states the spaying surgery was done through a flank incision. The accredited Mexican veterinarian signature, printed name and accreditation number are recorded. Then, the overseeing Mexican veterinarian signs, prints their name and records their accreditation number. The date of the spaying is recorded; spayed heifers can not be imported into the U.S. until after 21 days post spaying. Additionally, the spayed heifer cannot be imported to the United States any later than 180 days after spaying. The official blue ear tags are recorded in consecutive order, as evidence of tuberculosis testing.

If all of the above information is found to be correct, the animals are permitted to move to the border for exportation to the United States. The port-of-entry will receive the officially approved paperwork for a given lot of cattle prior to the cattle arriving. Once the paperwork has arrived and the cattle are there, the cattle are approved for exportation. At the San Geronimo, Chihuahua port-of-entry, the cattle are all inspected by a USDA veterinary staff. This inspection includes a tactile inspection to insure that no intact males or females cross the border into the United States. At the time each lot is unloaded, the USDA staff knows the sequential number series for the lot crossing. Each animal is checked that it falls within the correct sequence. After the individual inspection, the cattle are put through a dipping vat that rids the animals of ticks and other external parasites. After dipping, the cattle are walked across the "no-man's land" between the two port-of-entries, and enter the United States.

Chapter IV

Strengthening Identification of High-Risk Animals Using a Novel Identification Approach

INTRODUCTION

This research project was originally designed to identify and verify 10,000 head of imported Mexican feeder cattle. The cattle were to be identified (retinal imaged) at the port-of-entries (Santa Teresa, NM and Polomas, NM) and followed through the production chain, as they went from the port of entry to a stocker operation, and eventually, to the abattoir, assessing a new technology's ability to "validate animal identification to maintain the animal's identity when devices are lost or removed and to measure the accuracy of animal identification" using a high risk population of cattle.

While the number and type of cattle changed from the original project proposal, the objective did not. Within this project objective, consideration was given to the normal commerce and movement of imported animals. Beyond testing the ability of the Optibrand System to maintain animal identification, the feasibility of using the system with current production and importation methods was also considered. Measurements of feasibility include changes needed to existing structures to accommodate retinal imaging, changes to current processing protocols and additional animal handling, subsequent labor requirements and overall ease of use of the Optibrand System.

Cattle imported from Mexico have been identified as a potentially high-risk group for disease transmission into the United States. With recent outbreaks of bovine tuberculosis, the most probable source of infection has been roping steers (Beals, 2006). The management and useable life of these recreational (rodeo type) animals is much different than that of imported stocker or feeder cattle. They have a much longer useful life due to the nature of their use, are more likely to be intermingled with other livestock at events and are very transient and make many movements during a rodeo season.

Looking at all cattle imported from Mexico, recreation cattle pose the biggest risk – both in terms of disease transmission and the ability to adequately trace-back to the premises of origin. Recreational cattle have a much larger risk of 'losing' their ear tags compared to other populations. Tags are lost as animals change ownership, as these animals are often two to four years old before they lose their value as recreational animals. Ear tags are often removed (illegally), as the cattle are roped and utilized. The ear tags make the cattle's ears sore, and when they are sore the animals do not rope as well (England, 2007). Recreational animals are often bought in large groups by stock contractors. The contractor may use the whole group of cattle for a rodeo season or choose to lease or sell the animals to other contractors. At the end of the season, the original contractor has many options on how to market the animal. Some will go directly to a feedlot, some will be retained and used for another season – depending on the animal's performance and some will be sold to smaller contractors or backyard ropers for continued use. This variability in marketing and frequent changing of ownership makes it difficult to keep track of recreational cattle.

Another difficulty with recreational animals is the lack of uniformity for continued testing and animal health regulations between states and events (Qualls, 2008). There are 41 voluntary brand states, 1 mandatory brand state with the remainder having no regulations on branding animals for identification. The lack of uniformity between states leads many contractors and producers to become confused concerning requirements for animal movements. Adding to this confusion is that each state has its own separate requirements for additional testing once the imported animals reach the state of destination; some states require testing within 60 days of arrival and some require no subsequent testing. Most recreational cattle user groups (roping clubs etc), do not have set requirements, but follow the directives of the hosting state (Qualls, 2008). Therefore, our objectives were to determine if the Optibrand system can be used to maintain animal identification through the production chain for imported Mexican spayed feeder heifers.

MATERIALS AND METHODS

The Union Regional Ganadera De Chihuahua Inc. livestock port of entry (Santa Teresa, NM) was the site for animal enrollment and initial data collection. Feeder cattle were enrolled June, 2007, and recreational cattle were enrolled in January and February 2008. At the port of entry, current exportation practices do not require individual animal restraint. Once the animals cross into the United States, they are sorted, weighed and shipped, very few are individually handled due to the high volume of animals that cross though the facility on a daily basis.

Feeder Cattle. In June, 2007 two groups of light weight spayed feeder heifers (50 head with a pay-weight of 8350 ± 5kg; 52 head with a pay-weight of 13,800 ± 5kg) were enrolled in the study. Enrollment consisted of each animal undergoing a 'two-eye session' using the OptiReader®, during which both eyes were retinal imaged (RI), creating a total of 204 individual images. The green metal ear tag from the state of Chihuahua and the blue official import tag were manually keyed into the OptiReader and embedded with the RIs for each animal. In addition, each animal received a white metal study tag, with a 'O' and a four digit number, to signify that cattle were part of the Optibrand study. If a two-eye session was not feasible due to eye injury, a picture of the damaged eye was obtained. All cattle were group weighed before and after animal handling for enrollment and pay weights were recorded (See Table 2). After weighing the cattle were sorted and shipped to a feedlot in Northern Texas for growing and finishing. After enrollment, one spayed heifer died in the pens overnight, and the study compensated the owner for the loss of the animal (See Table 2).

At the Northern Texas feedlot, the animals' identities were verified at the time of re-implant in August, 2007. At the feedlot the enrolled cattle were kept in separate groups for feeding and put in feedlot pens with other cattle. At re-implant, the only visual identifier that identified study cattle from non-study cattle was the white metal study ear tag. After finishing, the cattle were scheduled for the local abattoir in Northern Texas.

At the abattoir, two expert retinal imaging technicians collected left eye retinal images on the spayed feeder heifers from the feedlot. The cattle were sent in two groups, depending on when the cattle reached the target marketing weight, in January 2008 and April 2008 respectively. The animals were retinal imaged after dentition examination but prior to hide removal (see Figure 6). At the time of retinal imaging, each carcass received a barcoded paper tag to identify the carcass. The barcoded tag was read by the OptiReader®, and the barcoded tag was then placed with the ear tags in a plastic bag at the time of removal. After each carcass had passed through the hot carcass scale, the ear tags were collected and cross-referenced with the bar code tag, and the ear tag number resulting from each bar code tag were linked with the retinal image, allowing ear tags to be linked with retinal images.

Recreational Cattle. In January and February, 2008, 935 lightweight Mexican recreational steers were imported into the United States for the recreational use. At the time of importation, the 935 steers were enrolled in the study, creating a total of 1,870 images. At enrollment each steer was retinal imaged in a two-eye session (both eyes), ear tags (green state tag and blue official import tag) were recorded and each animal received a 'O' horn brand on the right horn, near the base of the horn (see Figure 7).

All cattle were exported by one Mexican stock contractor into the United States, and the exporter was able to give destination details for the cattle. During the course of the enrollment at the border, one animal had horn damage, and the owner was paid per the agreement signed by all parties involved in the study. After entering into the United States, the cattle were destined for five owners in three U.S. states. Three of the five owners were agreeable to having the study continued on their cattle in Arizona, California and Colorado. Due to unwillingness of the other owners to collaborate, cattle sent to the other owners were not traced as part of this study, beyond enrollment at the port-of-entry.

Colorado. Three hundred of the original 935 animals were sent to a location in Bennett, CO (outside of Denver, CO). On two separate visits, 78 cattle were verified for identity, retinal images were collected as well as blue import tags. These cattle were required to be bovine tuberculosis tested within 60 days of entry into the state, under Colorado law. At the time of verification, silver bovine tuberculosis test tags, as required by Colorado, were recorded as an animal identifier. The facilities were designed for branding and de-horning cattle, so additional light control was needed. On the initial visit, no light control was available, and only a few animals were retinal imaged, as image quality was poor without light control and the time to acquire images was increased as the animal's eye reacts to ambient light. Ambient light causes the animal's pupil to constrict, thus creating a smaller "window" into the eye and making it more difficult to capture high quality photographs of the retinal vascular pattern. On the second visit, light control was supplied by the researchers. Restraining each animal came to be difficult, as extra precaution was taken not to break horns, making it difficult to head-catch each animal. After a trial and error period, the best method for restraint was blocking the steer in the chute and manually restraining the head so that ear tags could be read and retinal images obtained. Cattle handling facilities proved to be a limiting factor throughout the project, as the horns on recreational cattle make it difficult to process these animal through chutes.

Arizona. Approximately 160 recreational animals were sent to the Phoenix, AZ area, to two producers. One producer located in the Phoenix area allowed the research team to visit and verify 34 animals. At this particular operation, the Arizona contractor leases a majority of his cattle to other smaller contractors, and the remaining cattle are out on native pasture until they are needed for the rodeo season. When the research team visited in April, 2008 the contractor was shipping 'spent' steers (those who were no longer useful as recreational animals) to a feedlot. In addition, the fresh steers enrolled in the project had been gathered for a movement to a different pasture. Prior to movement, cattle were retinal imaged (using a two-eye session) and all blue import and green Mexican state tags were recorded.

California. Two hundred fifty recreational animals were sent to Santa Margarita, CA after importation. In April 2008, 119 animals at the California location were processed for identity verification. At the time of verification, each animal was retinal imaged using a two-eye session, blue official import tags and green Mexican state tags were also recorded.

At this location, animals were being collected from pasture to be sold to other contractors. Working facilities at this location were well designed to process cattle with horns, and allowed for ideal individual animal restraint. Individual animal restraint using the de-horning chute (See Figure 7), allowed for quality retinal images to be collected, aided in the tag reading process and decreased the processing time required.

The cattle traveled between 380 to 1,120 miles to get to their respective destinations from the port of entry. The travel time ranged from 6 hours to over 16 hours depending on location. These numbers tell only part of the story, as the cattle did not originate at the border, and traveled many miles and hours to reach the port of entry.

After image collection retinal images are uploaded to the provided data management software (DMS) that allows for record management and one-to-one image comparisons. The image comparisons use a computational algorithm to assign each comparison a score. The matching algorithm assesses the degree of similarity between vessel size, vessel position and branching angles between retinal vascular pattern pairs. Each comparison is given a numerical score between zero and one hundred. The higher the score, the more likely the images in the comparison were from the same eye (Allen et al., 2007). This matching system has been designed to help sort large data sets, and aid in selecting candidates for visual inspection. To determine candidates for visual inspection, an inspection threshold can be assigned using the distribution of the algorithm scores (Allen et al, 2007). Matching scores between pairs that meet but do not exceed inspection threshold can be visually inspected to make a final determination of the match.

The remaining 225 animals that were enrolled at the border were sent to locations where producers were not able to be contacted or were not agreeable to the research team come and re-establish identity on animals in their possession.

RESULTS

The requirement for individually restraining each animal to collect retinal images was outside of the port's standard procedure and considerably changed the time required before animals could be shipped. The time to acquire images is largely impacted by the surrounding environment. First, animals have to be correctly restrained, ideally allowing the technician easy access to the animal's eye with ample space to adjust the angle of the camera for correct positioning. Secondly, light control must be adequate to keep the pupil dilated to allow satisfactory imaging. The facilities at the Santa Teresa port are open and well designed for cattle movement and restraint for typical processing. The modifications needed for light control would be fairly significant; enclosing the northern portion of the building could provide enough light control for retinal imaging. Portable temporary shade could be added, but the longevity and practicality of a temporary solution for an on-going process is questionable. At this time, requesting that the port of entry change the animal handling facilities without a change in importation procedures would be futile.

Any handling of cattle on the United States side of the port of entry will significantly increase the processing time for importation. The additional handling resulted in an increased need for labor and use of facilities that are usually not occupied. Any processing, movement or handling of animals contributes to stress on the animal and subsequent shrink. The study animals were weighed pre and post-handling for study enrollment. Calculations in Table 2 indicate that the loss per animal varies between $11.50 and $18.80. However, these numbers should be considered with caution, comparison with 'typical' shrink calculations for similar processes at the port of entry can not be made, as animal are typically not weighed on the Mexico side of the border. They are weighed only once at the U.S. port of entry to collect weight for payment before shipment.

Table 2. Cost calculations for feeder cattle processing at Santa Teresa border crossing at the time of importation to the United States.			
	50 hd (3cwt)	**52 hd (4cwt)**	**Heifer†**
Pay-weight (lbs)	18,410	30,425	-
Re-weight (lbs)	17,930	29,610	-
Total Loss (lbs)	480	815	369.00
Loss per hd. (lbs)	9.60	15.67	-
Price/lb ($)	1.20	1.20	1.20
Loss per hd. ($)	11.52	18.80	422.80
Shrink (%)	2.61	2.68	-
Total Cost due to Shrink and Animal Loss			$1976.40

†Compensation for heifer lost during study enrollment, included in calculation for processing.

Feeder Cattle. At enrollment in June 2007, the average time for processing was approximately 1m:59s ± 4m:58s (See Table 3), including restraining, tag cleaning and reading, retinal imaging and insertion of white metal study ear tag, the time required to capture the retinal images was 17.02s ± 18.93s (See Table 3).

Table 3. Animal handling event times and average time to acquire images during processing to obtain retinal images on feeder and recreational cattle imported from Mexico to the United States			
	n	**Animal Handling Event† (m:s)**	**Image Acquirement (s)‡**
Feeder Cattle Enrollment	102	1:59 ± 4.58	17.02 ± 18.93
Feedlot Verification	97	1:59 ± 6:25	10.15 ± 9.54
Recreational Cattle Enrollment (Jan)	288	02:13 ± 6:20	17.03 ± 18.17
Recreational Cattle Enrollment (Feb)	715	01:05 ± 0:57	10.57 ± 9.34
Colorado Verification	78	2:33 ± 1:32	37.75 ± 31.08
Arizona Verification	34	2:24± 1:31	21.4 ± 19.5
California Verification	129	2.09 ± 1.10	17.01 ± 17.73

†Animal Handling event calculated as time from head catching one animal until the next animal is in the head catch

‡Image Acquirement is calculated as total time the handheld video camera is functioning to record retinal image; Image Acquirement time is included in an Animal Handling Event

At the feedlot, the only method of identifying study cattle from their pen mates was the white metal study tag. For these cattle, there was a high rate of necrosis of the white ear tags due to incorrect placement of the tag (See Figure 5). For proper insertion, metal ear tags must go on the perimeter of the ear, and leave ample room for ear growth (especially in growing animals). This necrosis made study tags hard to see and difficult to find. For the tags that were easily visible, many of them had lost their paint, making them hard to read. These difficulties led to three animals not being retinal imaged at the feedlot. The identity of these cattle was verified using retinal images collected at the feedlot compared to those initial retinal images from enrollment. The 'one to many' matching resulted in 102 x 2 x 98 x 2 image comparisons, with each of the two retinal images collected for each animal at the feedlot (98 animals) being compared to each of the retinal images collected during the initial enrollment at the border (102 animals). For each eye, the one to many matching system reports the image comparison that has the highest match score, as well as the matching score for each comparison. The average matching score for verification from feedlot to enrollment was 95.54 ± 8.44 for Eye 1 and 97.12 ± 6.20 for Eye 2 where Eye 1 is the first eye that is imaged and Eye 2 is the eye that is photographed second. In the one to many matching analysis, 96 of 97 of Eye 1s were correctly matched with the highest score, and 94 of 97 Eye 2s were correctly matched and received the highest matching score for that comparison. At the time of enrollment, 1 animal had eye damage to Eye 1 that prevented the photographing the retinal vascular pattern. Three animals had eye damage to Eye 2 that prevented photographing the retinal vascular pattern. In each of these cases, the exterior of the eye was photographed. These four non-retinal vascular pattern photographs were the only images that could not be correctly matched using the one to many matching process.

In January and April, 2008 the spayed heifers went to harvest. Of the 96 animals that had image collection at both the feedlot and the harvest facility, 22% lost the blue official import tag; 20% lost the Green Mexican state tag and an additional 6% of the animals lost all forms of identification (See Table 4). Additionally, two animal's identifiers were not recovered at the hot carcass scale, but their identity was later verified using retinal images collected at harvest compared to images collected at enrollment.

Table 4. Ear tag loss in imported spayed Mexican feeder heifers from a U.S. feedlot to harvest in the U.S.

	Missing Ear Tags		
n	Blue Official Import Tag	Green Chihuahua State Tag	Both Blue Official Import and Green Chihuahua State Tags
96	22 (23%)	19 (20%)	6 (6%)

*3 animals did not have ear tag loss data collected at the harvest facility, animals omitted from analysis
*4 animals did not have ear tag loss data collected at the feedlot, animals omitted from analysis

Images collected at harvest were compared to: a) enrollment images and b) images collected at the feedyard. The 'one-to-many' comparison for enrollment to harvest images resulted in 2 x 102 x 98 comparisons, the 'one-to-many' feedlot to harvest comparison resulted in 98 x 2 x 98 comparisons. If each animal had four images collected for each animal, there will be an equal number of comparisons that should match and images that should not have matched. This creates a bi-modal distribution: the lower mode score resulted from comparisons that should not match (enrollment Eye 1 compared to feedlot Eye 2 on the same animal; enrollment Eye 2 compared to feedlot Eye 1 on the same animal), the higher mode score is the result of comparisons that should match (enrollment Eye 1 feedlot Eye 1 on the same animal; feedlot Eye 2 to enrollment Eye 2 on the same animal) and the score can not exceed a value of 100. Variance in the score values make possible that a certain amount of ambiguous score values will result from either low scores for image pairs that should match or high scores for image pairs that should not match. Under normal conditions, all ambiguous scores can be resolved by visual inspection. Comparing images from enrollment to harvest, 57 image comparisons received the highest match score from their true match. A true match is considered to be when a comparison is made between two unique images from the same eye from the same animal, a matching comparison was determined to be the 'true match' when the match score was cross checked against recorded ear tag data or a 'one-to-one' comparison of the images. Thirty five images did not receive the highest match score from their true match, but the true match was established. If the true match received the highest score, the average match score was 81.47 ± 18.0. For those true matches that did not receive the highest score that average was 52.38 ± 12.35 for the actual match. This low average score could be contributed to the number images with poor quality collected at the abattoir and feedlot. Comparing the enrollment images to the harvest images, 67 images received the highest match score for the true match, while 29 images did not receive the highest match score for the true match. For those that did have the true match as the highest match score, the average match score was 81.75 ± 13.11; the images that were true matches but did not receive the highest score had average match scores of 50.06 ± 12.55 (See Table 5). Average image acquirement time for images collected at the abattoir was not calculated, as image acquirement time was dependent on chain speed for the facility.

Table 5. Image comparison match score results for imported Mexican spayed feeder heifers.				
	Feedlot to Enrollment⁺		Harvest to Feedlot‡	Harvest to Enrollment*
n (total number of comparisons)	39,168		18,816	19,002
	Eye 1	Eye 2		
No. High Match score is True Match[a]	96/102 (94%)	94/100 (96.9%)	57/96 (59%)	67 (69.8%)
No. Matched, Top score NOT correct[b]	0	0	35/96 (36%)	29/96 (30%)
Average Score if Correct[a]	95.54 ± 8.4	97.14 ± 6.2	81.47 ± 18.0	81.75 ± 13.1
Average Score if Match found, Not Highest Score[b]	-	-	52.38 ± 12.4	50.06 ± 12.6

⁺At enrollment 2 animals did not have retinal images collected on both eyes due to eye damage
‡At the Feedlot 4 animals did not have retinal images collected
*At harvest 3 animals did not have retinal images collected suitable for matching
[a]Comparisons in this category had the true match receive the highest match score
[b] Comparisons in this category did not have the true match receive the highest matching comparison score, but a true match was found.

Recreational Cattle. In January 2008, the average animal handling time was 2m:13s ± 6m:20s, in February 2008, with a professional processing crew the average animal handling time was 1m:05s ± 57s. The additional time and labor required to enroll the recreational cattle varied from \$4 to \$11 (U.S.) per head (See Tables 6 and 7). Cattle with horns are more difficult to head catch, and restraint success is dependent on the skill of the chute operator. According to the contract agreement, all cattle with broken horns were compensated for by the project.

Table 6. Estimated processing costs in January 2008 for recreational cattle during study enrollment.				
Item	**Date**	**Quantity**	**$/hd Processing Fee**	**Total**
Processing				
	1/22/08	136	10.00	1360.00
	1/23/08	99	10.00	990.00
	1/24/08	53	10.00	530.00
Yardage				
	1/23/08	53	1.00	53.00
Total				$2933.00

Table 7. Estimated processing costs in February 2008 for recreational cattle during study enrollment.				
Item	**Date**	**Quantity**	**$/hd**	**Total**
Processing				
	2/19/08	171	10.00	1710.00
	2/20/08	422	10.00	4220.00
	2/21/08	122	10.00	1220.00
Yardage				
	2/19/08	515	1.00	515.00
	2/20/08	515	1.00	515.00
	2/21/08	120	1.00	120.00
Damages*				500.00
Total				8800.00

*Compensation for steer with broken horn due to processing for enrollment.

Colorado. At the Colorado location, 3 animals lost their official import tags. While this amount of loss is low, the rodeo season had yet to begin, and with that the reported need to remove ear tags for animal comfort and performance had not begun (See Table 8). The average animal handling time over two sessions was 2m:33s ± 1m:32s, with the average time to acquire images 37.75s ± 31.08s. Comparing the Colorado cattle to enrollment images created a total of 291,720 images. With the Colorado verified cattle, 50 (64.1%) animals had both true matches receive the highest match score, with an average match score of 98.6 ± 2.9. For those that only had Eye1 (8 animals, 10.26%) as the true match and the top score, the average true match score was 95.3 ± 6.7, for those that had Eye2 (2 animals, 2.56%) as the true match with the top score the average true score was 95.2 ± 7.1. An additional 18 animals were verified but neither Eye1 or Eye2 received a top score, for this group the average score of the true match was 59.2 ± 6.5. Using a matching score of 85 points as a limit for image verification, 8 true matches would have been labeled as non-matches (false negative) (See table 9).

Table 8. Ear tag loss at verification of imported Mexican recreational cattle after first change of ownership within the United States.

Location	No. Head Reported to Location	No. Head Verified	No. Official Blue Import Tags Lost	No. Official Green Chihuahua State Tags Lost
Arizona	110	37	1	1
California	250	129	2	2
Colorado	200	78	3	*

*Data not collected for Mexican State Tag on Colorado Cattle

Table 9. Matching comparison score results for imported Mexican recreational cattle located in Colorado.

	Total	% Group	Average Score
Both Eyes Highest [a]	54	76.1	95.8 ± 5.9
Eye 1 Highest Score Only [b]	9	12.7	98.5 ± 2.7
Eye 2 Highest Score Only [b]	5	7.0	96.9 ± 4.4
Neither Highest Score [c]	3	4.2	62.4 ± 9.5
False Negatives [d]	8	10.26	-

[a] When matching algorithms were computed, animals in this category had highest matching scores that accurately indicated a correct match for retinal image from both eyes when comparing enrollment images and those taken at their new locations.

[b] When matching algorithms were computed, animals in this category had the highest comparison matching scores that accurately indicated a correct match for either Eye 1 or Eye 2

[c] When matching algorithms were computed, animals in this category had neither Eye 1 or Eye 2 accurately matched with the highest match score.

[d] When matching algorithms were computed, animals in this category had their true match receive less than 85 points, forcing them below the inspection threshold of 85 points, where comparisons below 85 points are assumed to non-matches without individual visual inspection.

The cattle residing in Colorado will travel between events in Colorado, Wyoming and Kansas. Some cattle will be sold to smaller contractors and roping clubs after their first season. The contractor has arrangements with a local feedyard to finish the cattle once their recreational use is over, and cattle will go to an abattoir in Colorado.

Arizona. Of the 110 the owner had received in Arizona, 34 were available for retinal imaging. Of this 34, 1 had lost a Mexican state tag, and an additional animal had lost the blue import tag (See Table 8). The one to many match for the Arizona cattle to resulted in 127,160 comparisons (935 x 2 x 34 x 2). The average match score for the Arizona cattle 44.1% (15 animals) had both eyes receive the highest score for the true match, the average score was 92.07 ± 11.6. Three animals had Eye1 correctly matched with the true match as the highest score with an average match score of 83.02 ± 9.1. An additional seven animals had Eye2 only correctly matched with the true match receiving the high score with an average true match score of 92.07 ± 7.9. Nine animals had neither eye assigned the true match with the highest score, this group averaged a true match score of 58.2 ± 4.3 (See Table 8). Using an 85 point threshold for matching, nine animals would be labeled as not-matching, when in fact, the true match was given a score lower than 85 points. The average animal handling time was 2m:24s ± 1m:31s, and the average time to acquire the retinal image was 21.4s ± 19.5s (See Table 3).

Table 10. Matching comparison score results for imported Mexican recreational cattle located in Arizona

	Total	% Group	Average Score
Both Eyes Highest [a]	15	44.1	92.2 ± 7.9
Eye 1 Highest Score Only [b]	3	8.8	93.9 ± 5.6
Eye 2 Highest Score Only [b]	7	20.6	83.0 ± 10.5
Neither Highest Score [c]	9	26.5	58.2 ± 4.3
False Negatives [d]	9	26.5	-

[a] When matching algorithms were computed, animals in this category had highest matching scores that accurately indicated a correct match for retinal image from both eyes when comparing enrollment images and those taken at their new locations.

[b] When matching algorithms were computed, animals in this category had the highest comparison matching scores that accurately indicated a correct match for either Eye 1 or Eye 2 only.

[c] When matching algorithms were computed, animals in this category had neither Eye 1 or Eye 2 accurately matched with the highest match score.

[d] When matching algorithms were computed, animals in this category had their true match receive less than 85 points, forcing them below the inspection threshold of 85 points, where comparisons below 85 points are assumed to non-matches without individual visual inspection.

Cattle were verified at a set of shipping pens located on the allotments the cattle are pastured on. With the help of a qualified chute operator, each animal was individually restrained making retinal imaging and ear tag reading easier. Temporary light control was provided by the researchers, in the form of a portable tent. This allowed for adequate light control for retinal imaging.

Spent recreational cattle from this particular Arizona producer, if not sold or contracted to a smaller recreational user, will be sent to a feedlot. At the time of verification, spent cattle were being sent to feedlot in Texas for finishing before going to the abattoir. The cattle destined for the abattoir had the tips of their horns removed, to lessen the likelihood of injuries and bruising in the feedlot.

California. Of the 129 animals that were verified at the California location, 2 had lost blue official import tags, and an additional 2 animals had lost green Mexican state tags (see Table 8). It is important to note that animals were received from the port-of-entry and went directly on pasture. These tag loss numbers may not give an accurate depiction of tag loss, as the animals had not begun their life as recreational animals. The one to many comparison for the California cattle resulted in 445,060 comparisons (935 x 2 x 119 x 2). One hundred seven of the California cattle had both eyes true matches receive the highest matching score (89.92%), with an average matching score of 97.5 ± 2.9. Only Eye1 received the highest match score for four animals (3.36%), with an average true match score of 95.3 ± 6.7; Eye2 received the highest match score for six images (5.04%) with an average score of 95.2 ± 7.1. A small portion 1.68% (2 animals) had neither eye receive the highest score (See Table 9). Using an 85 point matching score as the cut-off for verification, five animals would have falsely been labeled as non-matches. The average animal handling time was 2m:09s ± 1m:10s, the average time to acquire retinal images was 17.01s ± 17.73s.

Table 11. Matching comparison score results for imported Mexican recreational cattle located in California

	Total	% Group	Average Match Score
Both Eyes Highest[a]	107	91.5	97.8 ± 4.48
Eye 1 Highest Score Only[b]	3	2.5	97.9 ± 1.83
Eye 2 Highest Score Only[b]	6	5.1	98.5 ± 2.37
Neither Highest Score[c]	1	0.9	70.3 ± 14.5
False Negatives [d]	5	4.2	-

[a] When matching algorithms were computed, animals in this category had highest matching scores that accurately indicated a correct match for retinal image from both eyes when comparing enrollment images and those taken at their new locations.

[b] When matching algorithms were computed, animals in this category had the highest comparison matching scores that accurately indicated a correct match for either Eye 1 or Eye 2

[c] When matching algorithms were computed, animals in this category had neither Eye 1 or Eye 2 accurately matched with the highest match score.

[d] When matching algorithms were computed, animals in this category had their true match receive less than 85 points, forcing them below the inspection threshold of 85 points, where comparisons below inspection threshold are assumed to be non-matches without individual visual inspection.

During their time as recreational animals, the cattle will travel to events throughout California, Nevada and into Utah. After their time as recreational animals, arrangements have been made for the cattle to be fed out in Idaho and then sent to the abattoir.

DISCUSSION

Results from these field trials are consistent with that of Allen et al (2007), and that identity was able to be established on all animals enrolled in the study. Animal identity could be re-established even with tag loss. The investigation threshold of 85 points generated a larger number of ambiguous match scores, but is less likely to falsely label a comparison as a match when it is in fact a non-match (false-positive). The investigation threshold should be adjusted depending on the severity of consequences of false-positives. In re-establishing animal identity in response to lost tags on a commercial operation the consequences of a false-positive would be much less severe than when re-establishing identity or verifying identity in the instance of an animal disease threat.

Feeder Cattle. Cattle that are imported into the United States specifically as feeder cattle are unrealistic candidates for using the Optibrand System as a means of identification. The cattle enter the U.S. and usually reside within U.S. less than a year before going to harvest, depending on movements and marketing decisions. Loss of identity within the typical imported feeder animal segments is not outside of industry standards, and the animals will likely make less than two major movements within their lifetime in the U.S. Limited movements and tag losses make these animals less likely candidates for retinal imaging for identification purposes. Additional processing for these animals is questionable at the time of importation as the handling animals will have an impact on shrink loss and final pay weight.

Recreational Cattle. Recreational cattle may pose the biggest threat with their transient nature, likeliness for multiple owners and multiple functions over a useful lifetime. The fact that recreational cattle move so often in varying groups to different events throughout the United States makes them a population of concern. Weight loss and shrink are not a concern for recreational animals, as they are sold on a per head basis, retinal imaging could provide a solution for recreational animal identification. Once again, expecting retinal imaging to take place on the United States port of entry is not reasonable. The retinal imaging could be incorporated in the processing procedures for exportation.

IMPLICATIONS

The research conducted in this study indicates that retinal imaging of imported Mexican cattle at the United States port of entry is not a feasible recommendation, based on the speed of commerce at the port of entry. In addition to slowing commerce the cost of processing these animals at the port-of-entry needs to be taken into consideration. The additional processing can make a significant change in pay weight, and the question remains: who should be responsible for the loss, the buyer or seller? With neither group likely to volunteer, that additional aspect could make commerce even more precarious. If retinal imaging is required for cattle entering the United States, it may be most feasible to expect retinal imaging to become a routine part of the processing taking place for exportation before arriving at the port-of-entry (bovine tuberculosis testing, branding, tagging etc.). Perhaps cattle intended to directly enter the stocker or feedlot systems are less of a threat than previously thought. With their limited movement and short lifespan in the United States, focus may be shifted to another population with better results.

CHAPTER V

EVALUATION OF OPTIBRAND OPERATOR TRAINING

INTRODUCTION

For a new method of identification to be accepted by producers and industry alike, it must be reliable and ultimately user friendly. Additionally, it must provide reliable, consistent results that can be adopted by a wide variety of personnel throughout the productions chain. The Optibrand System is a technology dependent method of identification that relies on operators' ability to consistently acquire quality images for future comparison.

If this technology is to be used throughout the industry, there would be a need for a large proportion of the workforce to be trained to use the Optibrand System. These trained individuals would be responsible for collecting quality images in a timely fashion that could be relied upon for future re-establishment of animal identification. The objectives of this project were to determine if newly trained operators can repeatedly collect quality images, can collect images that can be used to successfully re-establish animal identification, and can acquire images in a timely manner.

MATERIALS AND METHODS

To determine the degree of training required for newly trained operators to routinely capture satisfactory retinal images, a study was set up at Colorado State University using two new operators working side-by-side with an experienced operator. Novice operator 1 attended a standard two-day training session as typical for new operators within two weeks of study initiation, while Novice Operator 2 had been trained over 1 year prior to study initiation with intermittent use of the Optibrand system. After these sessions, both new operators met the qualifications to be Novice operators as defined in the Optibrand Operator Benchmarks (see Figure 8a,b,c).

Two groups of 48 feedlot animals (total of 96 head) were used for the training evaluation. The three operators each collected retinal images from both eyes of each animal, as well as the ear tag number during enrollment and a picture of the animal's ear tag during enrollment and a second verification session. Each novice operator's images from the verification session will be compared with the images from the initial session (enrollment). In order for an operator to gain competency, two factors must be taken into consideration: time to acquire the image and the quality of the image itself.

The retinal vascular pattern image collected by the Optibrand System is converted into a blob file that includes any information added to the animals file at the time of imaging. The blob files are transferred to DMS, to determine the 'image verification' score, a computational matching algorithm (See Figure 9). The matching algorithm assesses the degree of similarity between vessel size, vessel position and branching angles between retinal vascular pattern pairs. Each comparison is given a numerical score between zero and one hundred. The higher the score, the more likely the images in the pair were from the same eye (Allen et al., 2007). This matching system has been designed to help sort large data sets, and aid in selecting candidates for visual inspection. To determine candidates for visual inspection, an inspection threshold can be assigned using the distribution of the algorithm scores (Allen et al, 2007).

Matching scores between pairs that meet but do not exceed inspection threshold could be visually inspected to make a final determination of the match.

Time to acquire image is varies with benchmark levels, Novice operators retinal images from 25-50 animals with an average time of no more than one minute per eye; image verification scores should be greater than 85 points (inspection threshold) on every image submitted. To obtain 'Experience Operator' status, the individual must be able to retinal image 25-50 animals with an average of less than 30s per eye, with image verification scores greater than 85 point inspection threshold. To be an 'Expert Operator' the operator must be able to collect retinal images from 25-50 head with an average time of less than 15 seconds per eye, and have image verification scores greater than the 85 point inspection threshold.

Statistical Analysis. Data were analyzed using PROC MIXED to determine the difference in time between operators on the repeated measures taken on the feedlot animals. PROC MIXED was also used to analyze the data for the difference between operators and match scores.

RESULTS

An internal feature of the OptiReader® automatically tracks the length of time that the video feature of the camera is in operation for each image collected. The results show that the two, newly trained operators were able to collect images at less than 30s per eye, having them benchmarked as Experienced Operators. Novice Operator 1 was able to improve time to acquire images from 25.1 seconds per eye to 9.3 seconds per eye on the final verification. Novice Operator 2 was able improve from 24.7 seconds per eye to 18.3 seconds per eye (See Table 10). The retinal imaging process takes a considerable amount of hand-eye coordination, and a high degree of variability between operators is to be expected. Novice 1 was able to make more improvement with imaging speed, while Novice 2 made less improvement. However, both new operators' demonstrated satisfactory speed and both showed acceptable proficiency (See Figure 8 a,b,c).

Table 12. Training Evaluation – Average time required for each operator to collect an image of the retinal vascular pattern from a feedlot animal.

	Expert Operator (s)	Novice 1 (s)	Novice 2 Time (s)
Group 1 Enrollment (s/image)	10^{a}	25.1^{b}	24.7^{b}
Group 1 Verification (s/image)	12.2^{a}	19.5^{b}	21.5^{b}
Group 2 Enrollment (s/image)	10.2^{a}	22.9^{b}	22.9^{b}
Group 2 Verification (s/image)	9.3^{a}	9.3^{a}	18.3^{b}

[a,b] Row values with different superscripts differ ($P < 0.05$)

The ability of an operator to repeatedly obtain quality images that can be used for comparison is very important for successful use of a retinal imaging identification system. The operator must not only be able to collect a quality image, but must be able to collect a similar image to use for verification. The quality and amount of over-lap between images is what dictates the success of comparisons. The Expert operator had an average true match score of 97.42 ± 3.87, Novice 1 had a true match average of 97.63 ± 4.71 and the Novice 2 operator had a significantly different true match average of 89.13 ± 11.27 (P <0.05) (See Table 13). The Expert and Novice 1 operator each had over 99 and 97% of their true match score greater than 85 points, while Novice 2 had 71% of true match scores greater than 85 points. Expert and Novice 1 operators had less than 5% of their true match scores less than 85 points (1 and 3%, respectively) while Novice 2 had 29% of true match images score under 85 points. Concerning two eye sessions, the Expert operator had 92 animals (96.8%) of images receive 85 points or greater for both true match scores, Novice 1 had 92.6% (88 animals) and Novice 2 reported 56 (58.9%)animals with both eyes receiving the top match score. The Expert operator and Novice 1 had a true match score of 85 or greater on one or both eyes for every animal (100%), Novice 2 had 85.3% animals with a true match score of 85 or greater. Novice 2 was the only operator to report any animals with both eyes under the 85 point true match threshold, with 14.7% of images collected falling under this criteria.

Table 13. Operator training match comparison score results			
	Expert	**Novice 1**	**Novice 2**
Average Match Score [c]	97.42 ± 3.87[a]	97.63 ± 4.71[a]	89.13 ± 11.27[b]
No. Matches > 85 [d]	187 (99%)	183 (97%)	135 (71%)
No. Matches < 85 [e]	2 (1%)	6 (3%)	54 (29%)
No. Both Eyes over 85[f]	92/95 (96.8%)	88/95(92.6%)	56/95(58.9%)
No. with 1 or more eye match scores > 85[d]	95/95 (100%)	95/95 (100%)	81/95(85.3%)
No. with both eyes under 85[g]	0 (0%)	0 (0%)	14 (14.7%)

[a,b] Row values with different superscripts differ ($P<0.05$)
[c] True Average Match Scores for True Matches.
[d] Number of images that each operator had about the 85 point investigation threshold
[e] Number of true matches with a match comparison score less than 85 points
[f] Image comparison results in where the true matches for at least one eye from the animal resulted in comparisons greater than 85 points
[g] Image match comparison results where both eye comparisons resulted in a score less than 85 points.

DISCUSSION

Inexperienced operators have the ability to be taught proper technique and image collection skills. The findings from this study agree with the findings of Whittier et al. (2003), that there is a significant effect of operator on time to acquire images. While new technicians can be taught, this is a method of identification that is highly dependent on technology, and requires the technician to be technologically savvy for full use of the OptiReader® and DMS. The success of the Optibrand system is dependent on the technician's ability to competently capture retinal images that can be used for identity verification in the future.

APPENDIX: Figures

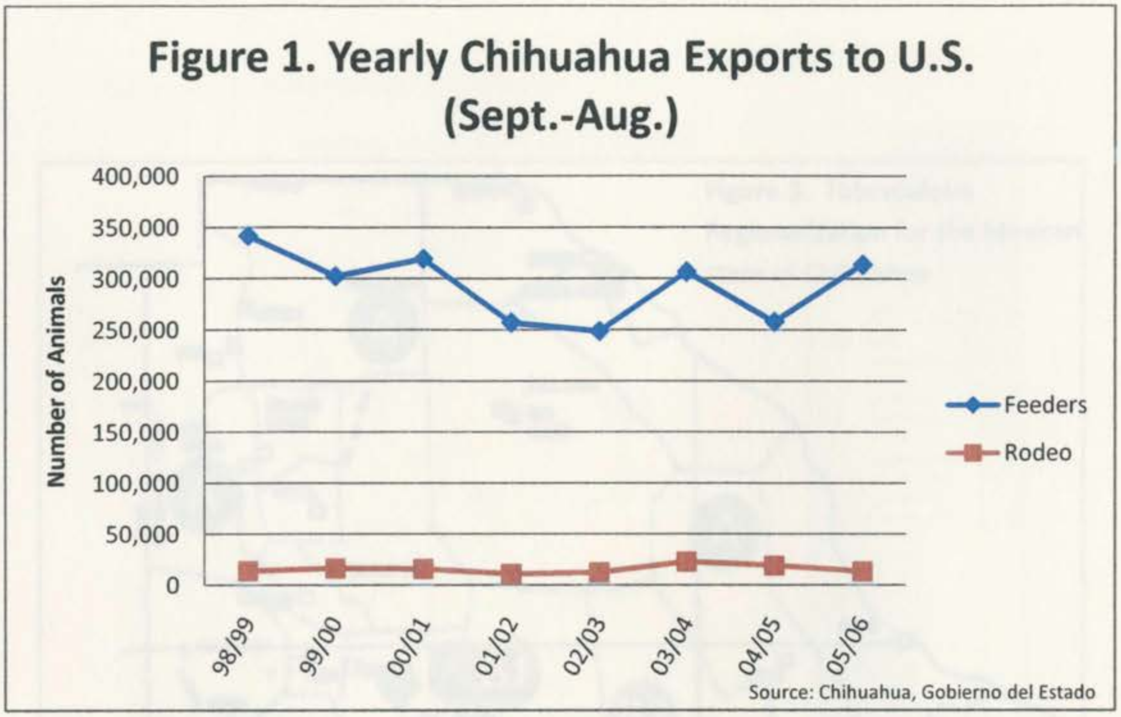
Figure 1. Yearly Chihuahua Exports to U.S.
(Sept.-Aug.)
Number of Animals
400,000
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
98/99
99/00
00/01
01/02
02/03
03/04
04/05
05/06
Feeders
Rodeo
Source: Chihuahua, Gobierno del Estado


Figure 2. Yearly Chihuahua Exports to U.S.
(Sept. - Aug.)
Number of Animals Inporter
25,000
20,000
15,000
10,000
5,000
0
98/99
99/00
00/01
01/02
02/03
03/04
04/05
05/06
Rodeo
Source: Chihuahua, Gobierno del Estado

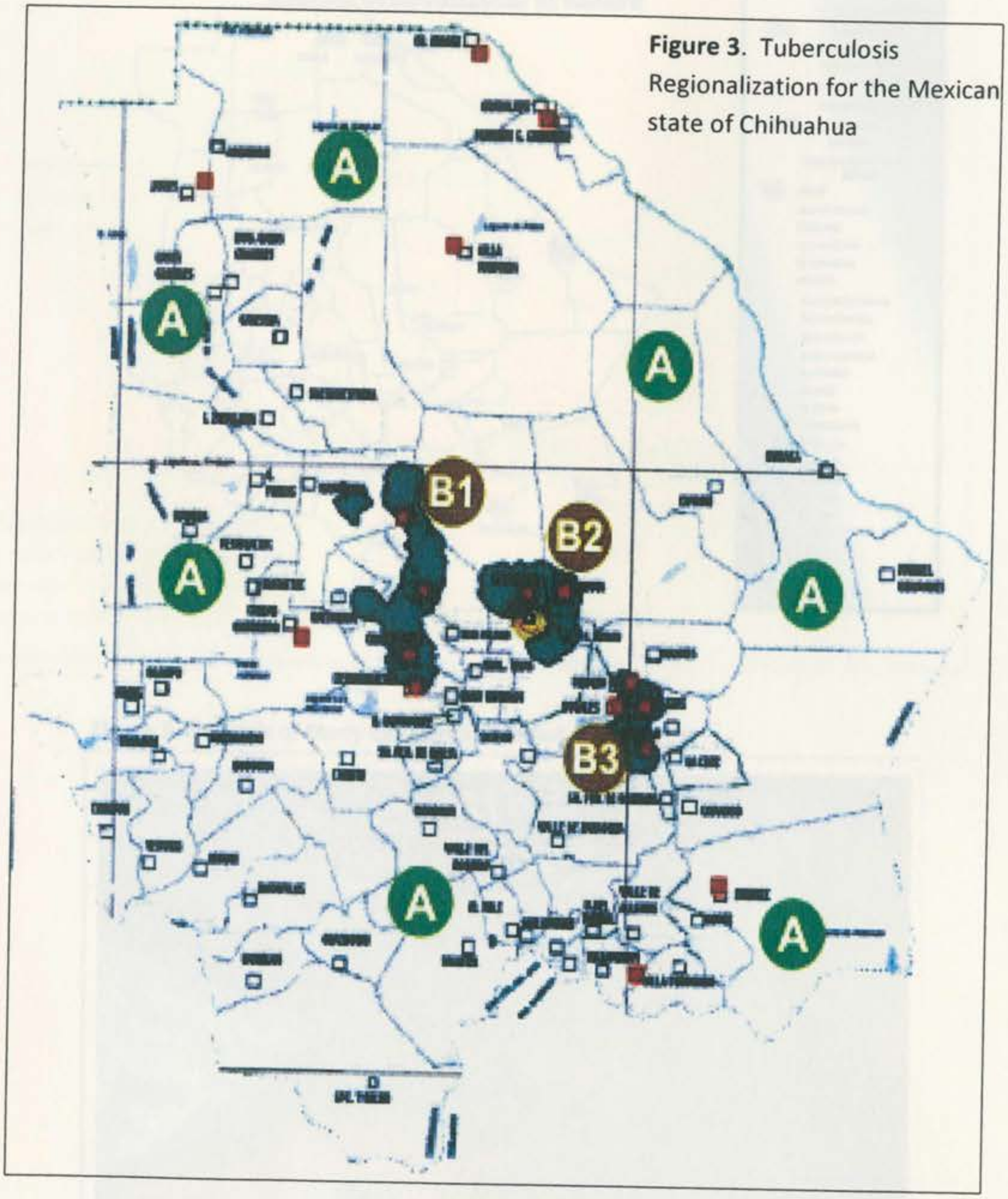

Figure 3. Tuberculosis Regionalization for the Mexican state of Chihuahua

Figure 4. Caseta Locations in Chihuahua

Figure 5. Necrosis of Study Ear Tags at Feedlot

Figure 6. Harvest Facility Diagram for Retinal Imaging

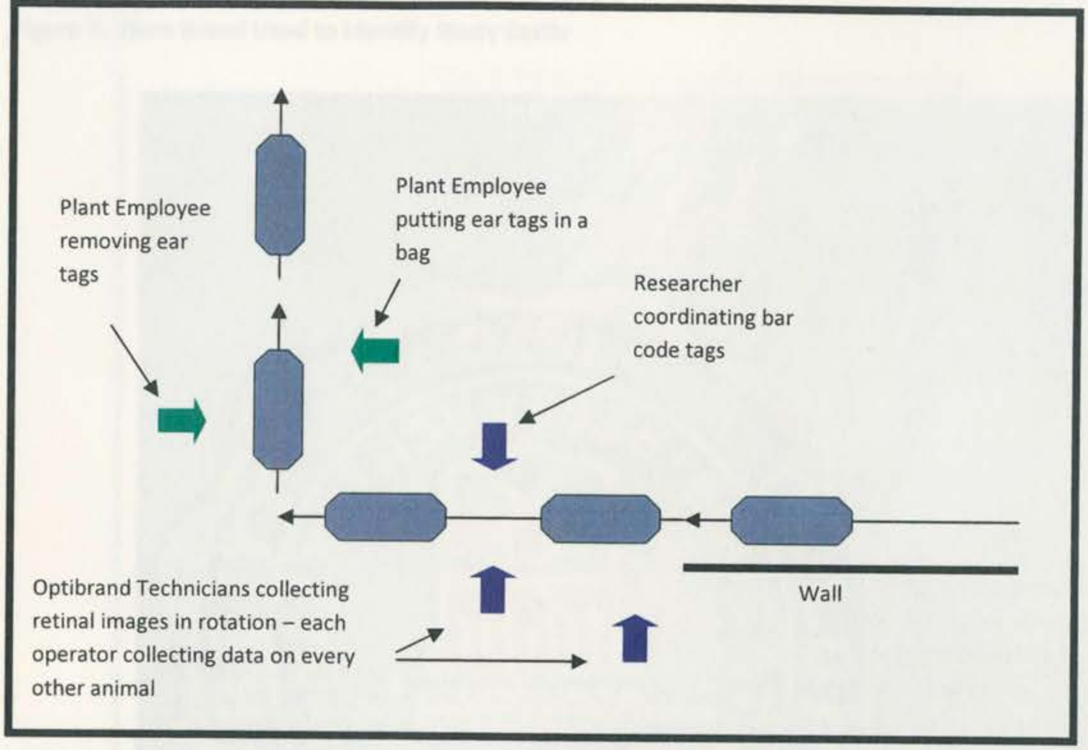

Figure 7. Horn Brand Used to Identify Study Cattle

Figure 8. Optibrand Operator Benchmarks

Optibrand Operator Benchmarks

Field Technicians - Retinal Imaging

Operator Benchmarks were established to evaluate and quantify the skill level of technicians trained to use the OptiReader® Device to collect images of the retinal vascular pattern (RVP) as a means of identifying livestock. To qualify for certification at each of the skill levels, operators must meet the qualifications listed below for accuracy and proficiency of RVP image collection. Based on these skill levels, technicians can set realistic expectations for the number of animals they will be able to enroll in one day.

Operator benchmarks are set at three skill levels: Novice Operator, Experienced Operator, and Expert Operator. At each level, operators must:

- Be able to capture a high quality image of the Retinal Vascular Pattern (RVP) from both eyes of each animal.
- Achieve the stated image collection proficiency as measured in average time to acquire images.
- Have a thorough understanding of how the OptiReader Device works.

As operators progress from Novice Operators to Experts, they will become more proficient in capturing data, and their time to acquire a retinal image will decrease. More experienced operators can expect to capture data on more livestock per session than a Novice Operator.

Novice Operators

Individuals desiring to use the OptiReader Device to collect the Retinal Vascular Pattern of livestock for identification and traceability should attend an Optibrand training session and should collect 25 – 50 sets of data (the RVP for both eyes on one animal constitutes one set of data). Operators should be trained on each species for which they will collect RVP images. At the end of the formal training, operators should be able to pass a written exam on basic OptiReader Device functionality. Operators may need additional practice after the initial training session to attain Novice Operator status.

Novice Operators should be able to:

- Collect RVP images from 25 – 50 animals with an average time to acquire images of less than one minute per eye. At this rate, the operator should be able to collect data on 10 to 20 animals per hour. Novice operators should expect to collect data for a maximum of three to four hours at a time and could expect to image 40 to 60 animals in one day.

Figure 8b. Optibrand Operator Benchmarks

Optibrand®

- Collect only high quality RVP images. Operators will collect duplicate sets of images from each animal. Image Verification of these images using Optibrand's DMS software must result in a score of 85 points or higher for each image collected.
- Demonstrate a thorough understanding of how the OptiReader Device works by using the device effectively to collect data in the field and by passing a written exam.
- Understand the importance of proper animal restraint and be able to make modifications to the system for animals that are more difficult to restrain properly.
- Understand the importance of proper light control and be able to make modifications to the facility to facilitate image collection.

<u>Experienced Operators</u>

Operators who have attended a 2 – 3 day training session and have collected data on over 100 head of cattle, sheep, and/or goats through the formal training and subsequent practicing should be able to achieve Experienced Operator status. Operators can use smaller groups of animals repeatedly to practice, but practice sessions should include as many different animals and types of facilities as possible.

Experienced Operators must be able to:

- Collect RVP images from 25 – 50 animals with an average time to acquire images of less than 30 seconds per eye. At this rate, the operator should be able to collect data on approximately 20 to 30 animals per hour. Experienced operators should expect to collect data for a maximum of four to five hours at a time and could expect to image 75 to 100 animals in one day.
- Collect only high quality RVP images. Operators will collect duplicate sets of images from each animal. Image Verification of these images using Optibrand's DMS software must result in a score of 85 points or higher for each image collected.
- Demonstrate a thorough understanding of how the OptiReader Device works by using the device effectively to collect data in the field and by passing a written exam.
- Understand the importance of proper animal restraint and be able to make modifications to the system for animals that are more difficult to restrain properly.
- Understand the importance of proper light control and be able to make modifications to the facility to facilitate image collection.

Figure 8c. Optibrand Operator Benchmarks

Expert Operators

Operators who have gained extensive experience may be able to achieve Expert Operator status. Expert Operators demonstrate the ability to collect retinal images on a wide range of livestock classes in a wide variety of situations. Expert operators have the ability to collect RVP images on up to 300 head of livestock in one day in facilities with adequate animal restraint systems and proper light control.

Expert Operators must be able to:

- Collect RVP images from 25 - 50 animals with an average time to acquire images of less than 15 seconds per eye. At this rate, the operator should be able to collect data on approximately 30 - 60 animals per hour. Expert operators should expect data for a maximum of four to six hours at a time. When collecting retinal images on large numbers of animals, it is recommended that operators switch off every two to three hours. Expert operators could expect to image 150 - 300 animals in one day.
- Collect only high quality RVP images. Operators will collect duplicate sets of images from each animal. Image Verification of these images using Optibrand's DMS software must result in a score of 85 points or higher for each image collected.
- Demonstrate a thorough understanding of how the OptiReader Device works by using the device effectively to collect data in the field and by passing a written exam.
- Understand the importance of proper animal restraint and be able to make modifications to the system for animals that are more difficult to restrain properly.
- Understand the importance of proper light control and be able to make modifications to the facility to facilitate image collection.

Figure 9. 'One to One' Matching Results