THESIS

BOVINE TUBERCULOSIS SLAUGHTER SURVEILLANCE IN THE UNITED STATES:

ASSESSMENT OF ITS TRACE-BACK FUNCTION 2001-2010

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ABSTRACT

BOVINE TUBERCULOSIS SLAUGHTER SURVEILLANCE IN THE UNITED STATES:

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The detection of gross bovine tuberculosis (bovine TB) lesions in cattle at slaughter and the successful trace-back to the herd of origin is critical to the detection of infected herds and for the progress of the national bovine TB eradication program in the United States (U.S.). A national animal identification system to identify and trace individual animals is currently under development in the U.S.; however, it is not yet fully implemented. In order to quantify the impact slaughter surveillance and traceability of bovine TB infected cattle has on the eradication of bovine TB from the cattle population in the U.S., this study was conducted with the aim to determine the ability of the current bovine TB slaughter surveillance system to trace infected cattle back to the herd of origin. Data obtained for the period 2001-2010, in which 386 bovine lesions were confirmed as bovine TB in the U.S., were used for this study. The specific objectives for this study were 1) to review and document the available literature related to the history of bovine TB control in the U.S., focusing primarily on the current method of disease detection (slaughter surveillance) and the impediments to eradication in the U.S., 2) to quantify the number of successful trace-backs of bovine TB infected animals to their herd of origin during 2001-2010 3) to quantify the number of trace-backs that found at least one bovine TB infected ("affected") herd, and 4) determine if selected factors were associated with the probability of successfully tracing infected animals and finding infected herds. The results of this study indicates that the odds of successful trace-backs are 7.06 times greater for cattle with official identification than without official identification (OR 95% CI: 1.66, 29.93, p-value =0.008). Additionally, the odds of successful trace-back are 15.47 times greater for adult cattle compared to fed cattle (OR 95% CI: 4.47, 53.48, p-value<0.001). Thus, application of official ID on all classes of cattle would increase the probability of successfully tracing bovine TB cases back to a herd of origin; however, under the current system it will not ensure a complete success in tracing bovine TB infected cattle to the herd of origin. While adult cattle are currently more likely to be traced back than fed cattle, it is worth noting that the effort and time required to find the herd of origin for both adult and fed bovine TB cases can be substantial and is highly variable. The results of this study provide an important tool to aid U.S.

officials in their decision making with respect to the evaluation and implementation of strategies for the national bovine TB control and eradication program.

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DEDICATION

This study is dedicated to the many individuals from the Animal and Plant Health Inspection Services (APHIS) and Food Safety Inspection Service (FSIS) who work diligently to help identify, trace and control bovine tuberculosis in the United States, as well as, to all the cattle owners, livestock markets and slaughter establishments who make their best effort to participate in the bovine TB disease control and eradication program.

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Introduction

Bovine tuberculosis (bovine TB), caused by the bacterium *Mycobacterium bovis (M. bovis)*, is a zoonotic disease with one of the widest host ranges of all pathogens (Grange & Collins, 1987). Cattle and other bovids are considered to be the reservoir hosts of bovine TB , but the bacteria can infect most mammalian species (warm-blooded vertebrates), including humans causing clinical disease (Morris *et al*., 1994; Corner, 2006, Kaneene & Pfeiffer, 2006; United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS)/Veterinary Services (VS), 2011g). Since the infection can be transmitted from cattle to humans, bovine TB is a zoonotic disease (Thoen & Barletta, 2006). Wildlife hosts have included the possum in New Zealand, badgers in Ireland and Britain, and cervids in the U.S.; spillover hosts have included humans, coyotes, pigs, dogs and cats (Brown *et al*., 1994; Corner, 2006). *M. bovis* is a slow-growing, gram-positive, rod-to-filamentous-shaped bacterium (Thoen & Barletta, 2004; USDA/APHIS/VS, 2011g). The survivability of *M. bovis* outside a host depends largely on the environmental conditions it is subjected too. The survival time of the bacterium can increase in moist environments and decrease in the presence of sunlight, low pH, other microbes, and rising temperatures (Morris *et al*., 1994).

The most infectious route of transmission, through which cattle become infected, is through aerosolized bacilli and inhalation via the respiratory tract, accounting for 80 to 90 percent of infections in cattle (Francis, 1947a; Pritchard, 1988; Griffin & Dolan, 1995, Menzies *et al*., 2000). In tuberculous cattle, the most commonly affected sites are the medial retropharyngeal lymph nodes, thoracic lymph nodes and lungs (Lepper & Pearson, 1973; Thoen & Himes, 1986; Whipple *et al*., 1996; Goodchild & Clifton-Hadley, 2001). Transmission can also occur through contact with saliva and other discharges if livestock share a common watering or feeding place with infected animals. The ingestion of contaminated milk is another important route for infection which can lead to infection presenting in the intestines and mesenteric lymph nodes (Morris *et al.*, 1994). In cattle, bovine TB usually takes several months to develop and can be transmitted as early as 10 days post-exposure but typically occurs 3 months post-exposure; however, the bacteria can lie dormant within a host without ever causing infection or resulting in the spread the bacteria (Neill *et al*., 1992; Morris *et al*., 1994, USDA/APHIS/VS, 2011g). During the course of infection, the

disease may progress rapidly or may be without clinical signs (USDA/APHIS/VS, 2011g). Postmortem

lesions are typically encapsulated and caseous (or calcified) granulomas (or tubercles); however, the size of

lesions is not an indicator of infectiousness (USDA/APHIS/VS, 2011g). In experimental infections, lesions

have been detected as soon as 14 days after exposure (Cassidy *et al*., 1998); however, under natural

conditions cattle in early stages of disease may have no visible lesions while producing substantial amounts

of aerosolized bacilli (Neill *et al*., 1992; Morris *et al*., 1994).

Control of selected infectious diseases, such as bovine TB, is usually performed by state government

programs, often in a working partnership with the farming industry. Control is desirable both from the

individual farmer's point of view and from the point of view of society at large. The problems posed by

bovine TB today are different from those faced nearly 100 years ago in North America. Initially the

impetus for public action came from the realization that the disease was zoonotic in nature and was a threat

both to the farming family and the consumer. Today, while the same human hazards remain, the risk to

human health is at an all-time low level in most developed countries due the introduction of milk

pasteurization in the early 1900's, the introduction of universal meat inspection programs and the success

of early bovine TB control programs. Consequently, the emphasis in controlling bovine TB has shifted

primarily to the trading implications of the disease for cattle and their products (Collins, 1999). Although

rare, bovine TB can still be a human health hazard in developed countries for people who consume

unpasteurized products and work in close contact with livestock. This scenario was seen in San Diego,

California, U.S., where a study revealed that 73 patients, mostly of Hispanic origin (Mexicans) presented

with microbiological evidence of *M. bovis* infection (Danker *et al*., 1993). Also, in New York City,

between 2001-2004, 1% (35 out of 4,524) of the human tuberculosis cases reported in New York City were

deemed to have been caused by *M. bovis*, and the most likely source of infection was consumption of fresh

cheese brought to NYC from Mexico (CDC, 2005).

Between 2005-2008, 128 out of 155 countries reported the presence of *M. bovis* infection and/or clinical

disease in their cattle populations (Michel *et al.*, 2010). In these particular countries, there are still serious

implications of dealing with bovine TB for those individuals who are farmers, slaughterhouse and rendering plant workers, government veterinarians and inspectors (Liss, *et al.*, 1994).

Typically, efforts to control and eradicate bovine TB (i.e., "test and slaughter policy") are focused at the national level and include a combination of the following methods (de la Rua-Domenech *et al.*, 2006; Olea-Popelka, 2007):

> 1) application of long-term, systematic programs of tuberculin skin testing and the removal of bovine TB reactors,
>
> 2) slaughter plant surveillance,
>
> 3) cattle movement restrictions,
>
> 4) repeat testing of infected herds, and/or
>
> 5) whole-herd depopulation.

National bovine TB eradication programs in countries in which the tuberculin test and slaughter policy have been adopted have proved highly effective at eliminating infection from infected herds, provided the policy was sustained at an effective level for a relatively lengthy period (i.e., at intervals as short as less than a year in the absence of wildlife reservoirs) after the clinical disease had all but disappeared (Morris *et al.*, 1994, O'Reilly & Daborn, 1995). Despite the inescapable limitations of existing diagnostic tests, bovine TB has been effectively eradicated from developed countries and regions with the implementation of sound programs of regular tuberculin skin testing and removal of reactors, in conjunction with slaughterhouse surveillance for undetected infections, repeat testing and culling of infected herds, cattle movement restrictions to prevent introduction of infected animals and occasional slaughter of entire herds (de la Rua-Domenech *et al.*, 2006). For example, Australia (in 1997) and several European countries have effectively eradicated bovine TB from the cattle in their national herd (Government Veterinary Journal, 2006; Queensland Government, 2009). Other countries however, have not had this level of success despite their ongoing bovine TB control campaigns. In the U.S., Mexico, United Kingdom, Ireland, Spain and Italy, results of bovine TB eradication campaigns in cattle have been mixed and the recurring presence of bovine TB within these countries continues to pose significant animal health problems and financial burden

(Government Veterinary Journal, 2006; United States Animal Health Association [USAHA], 2007a and 2007b; USDA/APHIS/VS, 2009a).

In the U.S., the Cooperative State-Federal Bovine Tuberculosis Eradication Program, was established in 1917 (Palmer & Waters, 2011) and is administered by the U.S. Department of Agriculture (USDA) Animal Plant Health Inspection Service (APHIS), State animal health agencies and U.S. livestock producers (USDA/APHIS/VS, 2011g). The original control program which emphasized routine (or systematic) area wide testing of cattle using the tuberculin skin test (and the removal (slaughter) of all reactors), resulted in the lowering of the initial prevalence in cattle herds of 5% in 1917 to less than 0.5% in every county by 1941 (USDA/APHIS/VS, 1990 and 2009; Olmstead & Rhode, 2004). Having this low prevalence in each county allowed the country to achieve "disease-free" attestation in 1941 (Hastings, 1942). From 1952-1954 the prevalence plateaued at a low of 0.11%, however, it escalated to 0.23% in 1959 (Anderson, 1959) around the same time the primary method of detecting disease changed from routine area wide tuberculin testing to slaughter surveillance (including epidemiological trace-back investigations of confirmed cases to their herd of origin). The exact year that the program shifted from routine area wide tuberculin testing to slaughter surveillance is disputed by different authors. Some reports indicated the shift occurred in the 1950's (USDA/APHIS/VS, 2009b) or 1959 (Frye, 1995; Gilsdorf *et al.*, 2006b), while other reports indicated it effectively occurred in the early 1960's (Meyer, 1988; Gilsdorf *et al.*, 2006a) or 1965 (Essey & Koller, 1994; Schoenbaum & Meyer, 1995; Whipple & Palmer, 2000). Under slaughter surveillance, the primary method used today to detect bovine TB in the U.S., the prevalence of the disease in cattle herds has decreased to an estimated 0.003% (or 0.3 per 10,000) in 1994 (Essey & Koller, 1994) and 0.001% (0.1 per 10,000) in 2009 (USDA/APHIS/VS, 2009d and 2009f); the prevalence in cattle has decreased to an estimated 0.00001% (0.1 per 1,000,000) in 2009 (USDA/APHIS/VS, 2009f). In the U.S. despite the control program's apparent success, affected herds continue to be identified, bovine TB remains a serious and costly disease of livestock in the U.S. (USDA/APHIS/VS, 2011g), and the livestock industry, farmers, and animal health officials continue to express dissatisfaction and concern with the persistence of this zoonotic disease in the national cattle herd. The continued presence of bovine TB at a national level precipitates expenses related to: inspection and condemnation of affected parts (or all) of animal carcasses,

movement restrictions on infected herds, trade implications for cattle and their products, and the general

costs of a national bovine TB control program (Olea-Popelka, 2007).

Study objectives

The purpose of this study was to determine the ability of the current bovine TB slaughter surveillance

system to trace confirmed bovine TB infected cattle back to the herd of origin, in order to quantify the

impact that slaughter surveillance of bovine TB infected cattle has on the eradication of bovine TB from the

cattle population in the U.S. To achieve our goal, we had four specific objectives. First, we reviewed and

documented the available literature related to the history of bovine TB control in the U.S., focusing

primarily on the current method of disease detection (slaughter surveillance) and the impediments to

eradication in the U.S. Second, we quantified the number of successful trace-backs of bovine TB infected

animals to their herd of origin. Third, we quantified the number of trace-backs that found at least one

bovine TB infected ("affected") herd. Fourth, we assessed if selected factors were associated with the

probability of successfully tracing bovine TB infected animals and finding bovine TB infected ("affected")

herds.

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Chapter 1 - Literature Review

1.1 History of bovine TB in the United States: Past and Present

1.1.1 Creation of the U.S. bovine TB control and eradication program

The creation of several U.S. organizations lead to the commencement of the national bovine TB control and eradication program in 1917: a veterinary division within the USDA in 1883, the U.S. Livestock Sanitary Association, also in 1883, and the Bureau of Animal Industry (BAI) in 1884 (Diamant, 1978; Palmer & Waters, 2011). In 1891, the BAI began inspecting meat for the Federal Government and some years later it pledged to follow the U.S. Livestock Sanitary Association's resolutions and recommendations related to bovine TB (Salmon, 1894; Palmer & Waters, 2011). Realizing that the disease was contagious and spreading the U.S. Livestock Sanitary Association passed a resolution in 1899 recognizing that the use of tuberculin was the best means for detecting bovine TB in live animals and recommending that the States should authorize methods to control the disease (Palmer & Waters, 2011). The Federal Government has continued to follow the recommendations prescribed by the U.S. Livestock Sanitary Association, renamed the U.S. Animal Health Association (USAHA), with respect to establishing and implementing livestock disease regulatory programs (Palmer & Waters, 2011).

The BAI required tuberculin testing of all imported cattle by 1900 and shortly thereafter the Federal Meat Inspection Act (FMIA) of 1906 proclaimed that all carcasses affected with bovine TB and showing emaciation should be condemned; however, compliance with the act was not absolute (Ditewig, 1916; Palmer &Waters, 2011). The percent of carcasses retained because of bovine TB concerns was 1.8 for 1906 to 1916 (Palmer & Waters, 2011). Also in 1906, in order to establish bovine TB free herds, the BAI began an experimental process of test and removal in Maryland, Virginia, and the District of Columbia where the States were annually tested over a period of 12 years and owners were compensated for the slaughter of reactors (Olmstead & Rhode, 2007; Palmer & Waters, 2011). Over 17,000 cattle were tested during this time period, decreasing the prevalence of positive reactors from 18% in 1906 to less than 1% in 1919 (Olmstead &Rhode, 2007; Palmer & Waters, 2011). The success of this initiative was noted and prompted Congress to provide funds to the BAI to create the Tuberculosis Eradication Division in 1917;

the Division aimed to eradicate bovine TB from the nation's dairy herds, authorized the payment of indemnities to help curtail the economic losses associated with bovine TB and addressed public health concerns, using the tuberculin test as the sole diagnostic tool (Meyer, 1988; Whipple & Palmer, 2000; Olmstead & Rhode, 2007; Palmer & Waters, 2011). As the program evolved, the Federal Government began matching the State indemnities up to a certain amount (Olmstead & Rhode, 2007). The actions prescribed by the Tuberculosis Eradication Division facilitated the cooperative State-Federal bovine TB control and eradication initiative. Today, the Cooperative State-Federal Tuberculosis Eradication Program, as it became known, is administered by the U.S. Department of Agriculture (USDA) Animal Plant Health Inspection Service (APHIS) State animal health agencies and U.S. livestock producers (USDA/APHIS/VS, 2011g).

1.1.2 Historical overview of the emphasis of the U.S. national bovine TB control-eradication program

1.1.2.1 Routine area wide tuberculin testing (1917 to 1960)

The Cooperative State-Federal Tuberculosis Eradication Program initially emphasized routine (or systematic) area wide testing of cattle with the tuberculin skin test and the removal (slaughter) of all reactors (USDA/APHIS/VS, 2005b and 2009d). Additionally, the initial program called for bovine TB eradication to be achieved using three approaches (Meyer, 1988):

> 1) Establishing accredited herd plans that would assure bovine TB free cattle. The plans provided requirements for tuberculin testing of herd members and replacements. If a herd adhered to the prescribed guidelines, its accredited free status was maintained.

> 2) Establishing a set of Uniform Methods and Rules (UM&R) to insure sufficient and consistent programs were implemented between States; and

> 3) Establishing provisions for adoption of tuberculosis free and modified accredited free areas based on reactor rates. Tuberculin testing of all cattle in a defined area, usually a county, was conducted during the early stages of the program in attempt to assess the extent of infection and to rid each county of the disease.

The UM&Rs, formulated by the U.S. Livestock Sanitary Association in 1917, outlined the concepts of the accredited herds and the accredited areas based on reactor rates from routine area wide testing (Kiernan,

1917; Frye, 1995). To become a tuberculosis-free accredited herd, producers agreed to regular tuberculin testing of the entire herd and to keep accurate and complete animal identification practices (Kiernan, 1917 and 1918; Palmer & Waters, 2011). If regular tuberculin testing of a herd deemed them to be a bovine TB-free accredited herd then the producer would receive a certificate of accreditation from the state authority and the BAI. The certificate was valid for one year and declared that bovine TB had not been present within the herd for two years (Palmer & Waters, 2011). Frye (1995) explained the certificate as "no animal affected with tuberculosis had been found on two annual or three semi-annual tuberculin tests or by physical examination". Tuberculosis-free accredited herds could ship cattle interstate with no further tuberculin testing (Palmer & Waters, 2011). If each herd in a particular area met the requirements of being a bovine TB-free accredited herd, where the number of reactors would not exceed 0.5% after one complete test of all cattle and the herd owners were in compliance with sanitary and disposal requirements, then that area would become a bovine TB-free area, known as a "modified accredited area" (Frye, 1995).

In general, the program moved from voluntary tuberculin testing to compulsory testing. Initially, conducting tuberculin testing was at the discretion of the farmer, at his own expense and indemnity was not paid for the slaughter of reactors (Palmer & Waters, 2011). Olmstead & Rhode (2007) explain that testing began locally with a majority of dairy cattle owners in a county agreeing to participate and test their herds. Next, if the area's cattle owners petitioned to implement a compulsory program or if a simple majority voted in favor of it in a special election, counties began compulsory testing cattle operators on an area wide basis. Eventually, the State's legislature enacted legislation requiring tuberculin testing of all cattle in a State (Meyer, 1988; Olmstead & Rhode, 2007). However, each State program differed as to whether they mandated the slaughter of all reactors, provided indemnity for each reactor removed and implemented strict importation regulations (Olmstead & Rhode, 2007). The quantity and frequency of the area testing evolved into tuberculin testing 15% of the cattle herds in each state each year so that "in theory during a 6-year accreditation period all of each State's cattle herds would have been tested at least once" (Essey & Koller, 1994). By 1921, forty-six states had eradication field offices and by 1922, all but six states were participating (Olmstead & Rhode, 2007; Palmer & Waters, 2011). The program was also extended by 1922 to include the following measures (Myers, 1940; Frye 1995):

1) the accreditation of the whole U.S. by states and within the states by counties,

2) the education and licensing of veterinarians so that they were adequately trained to carry out the

work and standards set by the BAI,

3) the continuing education of the public and law making bodies in all political divisions of the

country so that public funds would be available to compensate for this strict public health

measure,

4) the adequate compensation(s) to individual farms for the cattle slaughtered so that financial ruin

would not follow their cooperation with the Government's plans, and

5) a yearly compilation of all available figures on the results of the tuberculin testing to serve as a

guide for future plans as well as an estimate of the accomplishments to date.

Figure 1 summarizes the hierarchical nature of the initial program that relied on routine area wide

tuberculin testing, accredited herds and accredited States.

Conceptual Hierarchy of U.S. Routine Area Wide Testing

Entire U.S. declared Modified Accredited 1940-41

Modified Accredited States
All counties in the State have herd prevalence <0.5%*

Accredited Counties
Each county has herd prevalence <0.5%

Accredited Herds
Each herd has herd prevalence <0.5%
(less than 0.5% reactor rate)

Based on information in Hastings, 1942, Meyer, 1988, Olmstead and
Rhode, 2007 and Palmer and Waters, 2011

*Today States/zones with a bovine TB prevalence of less than 0.5% of
the total number of cattle herds in the State/zone are called Accreditation
Preparatory (AP) (USDA/APHIS, 2005a).

Figure 1: Conceptual hierarchy of the initial U.S. bovine TB control and eradication program utilizing
routine area wide testing, accredited herds and accredited States

The eradication program faced much resistance and controversy at upon its inception and implementation (Frye, 1995; Olmstead & Rhode, 2007; Palmer & Waters, 2011). Widespread hostility to compulsory testing was eminent as many individuals believed controlling the disease should be left in hands of herd owner, not the government. Additionally, the accuracy of the tuberculin test was widely challenged and disputed. Those adamantly opposing the State mandated testing included farmers, organized groups, individuals, and Senators across the nation. Rallies and protests, i.e. the Iowa Cow War, occurred, anti-campaign pamphlets and literature were created and disseminated, and court challenges ensued concerning milk ordinances, pasteurization, tuberculin testing, and the slaughter of infected cattle (Olmstead & Rhode, 2007; Palmer & Waters, 2011). Temporary injunctions were granted to the anti-test groups in the lower courts; however, the higher courts usually upheld the legality of the State's efforts (Olmstead & Rhode, 2007). Two different approaches for controlling bovine TB (once an infected herd was identified) were disputed: "test and segregate" versus "test and remove (slaughter)." The test and segregate approach, also known as Bang's model, after the Danish veterinarian Bernard Bang, involved separating and managing the affected herd in two groups based on the tuberculin test results: a healthy herd (consisting of non-reactors) and an infected herd (made up of reactors) (Norton, 1904). The approach aimed to increase the size of the healthy herd and decrease the size of infected herd by separating calves from their infected mothers at birth and feeding them pasteurized milk (Palmer & Waters, 2011). The test and remove approach, also known as test and slaughter, favored by most state/territory veterinarians, consisted of the slaughter of all animals reacting the tuberculin test (Palmer & Waters, 2011). States with laws mandating test and the slaughter of all reactors were challenged with a number of cases reaching the Supreme Courts in several states. The test and slaughter laws were upheld for each case with the reasoning that "the laws protected against disease and, under the common law, cattle infected with contagious disease were public nuisances and could be summarily destroyed by public officials without compensation to their owners" (Olmstead & Rhode, 2007; Tobey, 1894). Nonetheless, the indemnity payments provided in the late 20's and 30's increased participation and program support. Gradually, the public began favoring the national eradication scheme and controversy shifting away from compulsory testing and the efficacy of the test, towards costs and administrative details associated with the program (Olmstead & Rhode, 2007).

During the early to mid-twentieth century (1917-1960), the annual bovine TB prevalence was estimated based on individual-animal tuberculin skin test results (Gilsdorf *et al.*, 2006b):

Bovine TB prevalence = the number of cattle reacting to the tuberculin skin test
                          total number of cattle tested in the U.S. for a given year

The prevalence of cattle reacting to the tuberculin test decreased from an average of 5% in 1917 to 0.46% in 1940, as a result of the administration of 232 million nationwide tuberculin tests, which disclosed over 4 million reactors and lead to the destruction of 3.8 million cattle (Roswurm & Ranney, 1973; USDA/APHIS/VS, 1990). From 1917 to 1927, the number of accredited herds increased from 0 to over 96,000 (Kiernan, 1926). In 1941, with an overall bovine TB prevalence of 0.3% the entire U.S. was declared modified accredited for bovine TB and every county in the U.S. proclaimed modified accredited free prevalence of less than 0.5%[1] (Olmstead & Rhode, 2004). Having achieved this remarkably low prevalence in each county, the Secretary of Agriculture, at the time, declared, "the United States is now practically free of bovine tuberculosis" (Hastings, 1942).

The rapid reduction in bovine TB prevalence seen in the first half of the twentieth century was attributed to the stringent application of the test and slaughter method of control (Palmer & Waters, 2011). From 1948 to 1950, the U.S. bovine TB prevalence was maintained at 0.19% and from 1952 to 1954, 0.11% (11 reactors per 10,000 animals tested); however, in 1959, the prevalence doubled to 0.23% (23 reactors per 10,000 animals tested) (Anderson, 1959b; Frye, 1995). Anderson (1959b) mentioned that in 1959 one dairy state had an infection rate as high as 240 per 10,000 animals tested according to USDA monthly reports and the number of accredited herds in the U.S. in 1959 decreased to 52,946, in contrast to 275,000 in 1937. Despite the prevalence of reactors being relatively low at 0.23%, it is unclear how many total cattle were tested in the U.S. in 1959, as well as, the geographic area in which they were sampled.

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[1]*Today States/zones with a bovine TB prevalence of less than 0.5 percent of the total number of cattle/bison herds in the State/zone are called Accreditation Preparatory (AP) (USDA/APHIS, 2005a).*

Several possible reasons were cited for the increase in reactors in the late 50's and a slowdown in the eradication effort (Meyer, 1988; Anderson, 1959a and 1959b; Frye, 1995):

  1) shortage of manpower, veterinarians and supplies during World War II,

  2) increased movements of livestock,

  3) increased herd numbers and more concentrated livestock operations,

  4) delayed and decreased tuberculin herd testing, resulting in fewer accredited herds and failure of States to maintain the minimum requirements for county reaccreditation,

  5) reduced program funding,

  6) reduced public interest and public health concern (a shift in disease interest in favor of disease programs of higher priority),

  7) complacency on the part of the public, industry, herd owners and regulatory officials due satisfaction with the reaching the achievement goal (tendency for herd owners, counties and States do a little as possible to retain status).

1.1.2.2 Slaughter surveillance and epidemiological trace-back investigations (1960 to 2010)

The emphasis of the Cooperative State-Federal Tuberculosis Eradication Program shifted from routine area wide tuberculin testing to slaughter surveillance around 1960. There is however, disagreement among different reports regarding the exact year of the shift. The change in emphasis was noted to have occurred in the 1950's according to the USDA/APHIS/VS (2009c), in 1959 according to Frye (1995) and Gilsdorf *et al*. (2006b), in the early 1960's according to Meyer (1988) and Gilsdorf *et al*. (2006a), and in the year 1965 according to Essey and Koller (1994), Schoenbaum and Meyer (1995*)* and Whipple and Palmer (2000). Despite the lack of agreement regarding the date in which the emphasis of the bovine TB control and eradication program changed, for the last 40 years the primary method of detecting bovine TB and locating infected herds within the U.S. has been (Whipple and Palmer, 2000; USDA/APHIS/VS, 2005b):

  1) slaughter surveillance,

  2) including epidemiological investigation intended to find

        a) the herd of origin for lesioned cattle, and

        b) all possible source herds and exposed animals.

Ranney (1970) described the new approach of the program as: "We are giving considerable emphasis to the detection of tuberculosis on regular-kill meat inspection examination, tracing the animals that show lesions to their herd of origin and testing these herds." Frye (1995) explained "the main focus of the program was placed on tracing lesioned animals found by meat inspection at slaughter to their herds of origin and then emphasizing epidemiology to locate herds that have been exposed by the index herd or the source of infection." The main reasons cited for the change in approach to the emphasis on slaughter surveillance was that (farm-to-farm) routine area wide tuberculin testing was deemed inefficient and costly when the prevalence of bovine TB was low and tuberculin testing was considered an inefficient method to detect bovine TB when the prevalence was low (USDA/APHIS/VS, 1990; FSIS/APHIS, 1991; Kaneene *et al.*, 2006). Additionally, the implementation of an official backtag identification procedure around 1960 and was becoming increasingly efficient at identifying most slaughter cattle to their herds of origin (FSIS/APHIS, 1991).

For the mid-twentieth century (1950-60) through 2010, the annual bovine TB prevalence in the U.S. under the slaughter surveillance method was commonly estimated based on the number of confirmed bovine TB lesioned animals detected at slaughter (Gilsdorf *et al.*, 2006b):

Bovine TB prevalence = number of confirmed bovine TB cattle detected at slaughter
number of cattle slaughtered (and inspected) in the U.S. for a given year

The prevalence of bovine TB in cattle herds and animals in the U.S. has decreased over time under the slaughter surveillance method, the primary method used today to detect bovine TB in the U.S. For the period 1983-1990 (under the slaughter surveillance method), the animal-level prevalence of bovine TB in regular kill cattle by tuberculosis confirmed lesions detected was 0.0004% and in 1994, the prevalence of the disease in cattle herds (herd-level prevalence) in the U.S. was estimated at 0.003% (Essey & Koller, 1994). In 2009 (under slaughter surveillance), the prevalence of the disease in cattle herds was estimated at 0.001% (USDA/APHIS/VS, 2009d and 2009f) and the prevalence in cattle was estimated at 0.00001% (USDA/APHIS/VS, 2009f).

Despite the change in method to slaughter surveillance, bovine TB prevalence has continued to be reported largely as the as a reactor rate in animals or herds or non-specific as to the underlying method used to

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calculate the prevalence, versus being reported as the number of confirmed bovine TB lesioned animals detected. For example, Frye (1995) provided an estimated prevalence for 1967 and 1987 based on the reactor rate for all U.S. cattle tuberculin tested and USDA/APHIS/VS (2005b) provided an estimated prevalence for 2005 based on the results of area wide tuberculin testing. Gilsdorf *et al.* (2006a) provided the estimated prevalence of the disease in cattle in 2006 without describing the underlying method used to calculate the prevalence. Similarly, USAHA (2007a) and USDA/APHIS/VS (2009d) provided the estimated prevalence in cattle herds for 2007 and 2009 without describing the underlying method used.

Throughout the twenty first century, the number of affected herds found has been widely reported by Veterinary Services (VS) department of USDA APHIS. Of the ninety-two affected herds found during FY 1998 to 2009, 53 (58%) were beef, 26 (28%) dairy, 2 (2%) mixed use, and 11 (12%) captive cervid herds (USDA/APHIS/VS, 2009a). Naugle (2011) explained that approximately 10 affected herds are detected annually in the U.S. (Naugle, 2011). The number of bovine TB lesioned cattle detected at slaughter and the success in tracing these cattle back via epidemiological investigation to a herd of origin, source herds, and exposed animals is not something that is typically reported in government and scientific literature on the U.S. bovine TB control program.

1.2 Detection of Bovine TB cases

1.2.1 Bovine TB slaughter surveillance

Slaughter surveillance for bovine TB typically consists of post mortem examination, submission of any suspicious lesions (tissue) to a laboratory for confirmation of *M. bovis* via histopathology, polymerase chain reaction (PCR) and/or culture (Corner, 1994), and investigation to identify the herd of origin, source herd and exposed herds in order to control the spread of infection. The success of slaughter surveillance as a disease detection method is dependent on having the ability to detect bovine TB lesions and to determine where the lesioned animals came from.

1.2.2 Bovine TB slaughter surveillance in the U.S.

Since the 1960's, the primary means by which infected herds are identified in the U.S. under the bovine TB control and eradication program is slaughter surveillance and subsequent epidemiological investigations (Frye, 1995; USDA/APHIS/VS, 2005b and 2009d). As the number of cattle routinely tuberculin tested has decreased, the importance of slaughter surveillance has increased. Federal regulations require that States conduct routine slaughter surveillance in order for them to maintain their bovine TB status for cattle (USDA/APHIS/VS, 2009d). Routine slaughter surveillance is conducted under the combined responsibility of the Food Safety and Inspection Service (FSIS) and APHIS of the USDA. More specifically, FSIS is responsible for routine slaughter surveillance for conditions that render carcasses unsuitable for human consumption and APHIS is responsible for ante mortem bovine TB testing, necropsy and investigation of cases identified as bovine TB (Kaneene *et al.*, 2006).

Carcass inspection

Approximately 6,200 slaughter establishments in the U.S. are federally inspected (USDA, 2011b) and 27 States operate their own Meat and Poultry Inspection (MPI) programs (USDA, 2011c). The State MPI programs are required by law to be "at least equal to" federal inspection in terms of regulatory rigor (Kaneene *et al.*, 2006). Every cattle carcass, at FSIS regulated plants, is examined at slaughter for human consumption adequacy (Kaneene *et al.*, 2006). Human consumption adequacy is also referred to as the capability of use as human food which is defined by the Code of Federal Regulations (CFR), Title 9, Chapter 3, Part 301, Section 2 as any carcass or part or product of a carcass of any livestock, unless it is denatured or otherwise identified as required by the applicable provisions of Sections 314.3, 314.10, 325.11, and 325.13 to deter its use as a human food, or it is naturally inedible by humans (Code of Federal Regulations, 2010i).

At slaughter, during ante mortem inspection, federal and state meat inspection personnel observe presumably healthy bovines for abnormalities and signs indicative of health conditions or disease, like bovine TB, that would prohibit the animal from entering the food supply (USDA, 2011b). According to CFR, Title 9, Chapter 3, Section 309, Part 2, animals suspect of being affected with any disease or

condition that under part 311 may cause condemnation of the carcass on post mortem inspection are further examined by FSIS Public Health Veterinarians (PHV) who make case-by-case decisions on the disposition of the animal's condition and are handled in such as way as to retain its identity as a suspect until it is given final post-mortem inspection (CFR, 2010j; USDA, 2011b and 2011d).

For post mortem inspection, federal and state meat inspection personnel examine each carcass for signs of disease or pathological conditions that would render a carcass or part of a carcass unwholesome or unfit for human consumption. The federal and state meat inspection personnel are responsible for detecting lesions suspect of bovine TB and retaining the carcass until the FSIS PHV is summoned to perform further examination (USDA/APHIS, 2001b and 2011b). According to CFR, Title 9, Chapter 3, Section 310.3, the identity of every retained carcass and associated detached organ or other part must be maintained until the final inspection has been completed (CFR, 2010l) and Section 310.2 requires that all manmade identification, such as ear tags, backtags, implants, and other identifying devices, must be collected and remain with the carcass through viscera inspection in order to facilitate trace-back to the herd of origin (CFR, 2010k). Based on gross pathology and the guidelines in 9 CFR 311.1 'Disposal of diseased or otherwise adulterated carcasses and parts; general' and 311.2 'Tuberculosis', the PHV makes a decision on the disposition of the carcass (CFR, 2010m and 2010n). If upon inspection, a carcass has no generalized signs of disease or pathological conditions, then it is released and may enter the food supply and if there are any non-significant localized conditions, they are removed prior to the carcass entering the food supply.

Lesion submission

If evidence of bovine TB is found in the form a macroscopic granuloma (a nodular inflammatory lesion), it is shipped along with available animal identification and a completed USDA-APHIS-VS Form 6-35, "Report of tuberculosis lesions or thoracic granulomas in regular kill animals", to one of three laboratories for histo-pathology, PCR and bacteriological isolation. These laboratories include the National Veterinary Services Laboratory (NVSL), in Ames, IA, the FSIS Field Services Regulatory Laboratory in Athens, Georgia and the California Animal Health & Food Safety Laboratory System in San Bernardino, California (USDA/FSIS, 2009b and California Animal Health & Food Safety Laboratory System, 2011). Bovine TB

granulomas in cattle are often found in the lymph nodes of the head, thorax and/or abdomen (USDA/APHIS/VS, 2005b).   To diagnose the lesions as compatible with Mycobacteriosis, histo-pathological examination is conducted at NVSL (USDA/APHIS/VS, 2009c; USDA/FSIS 2009a); histopathology can be completed within 24-48 hours (Winblad and Duchek, 1973; Collins *et al*., 1985; Thomson, 2006, USDA/FSIS, 2009a). The histopathology results are reported to the veterinarian who performed the post mortem exam in order to make a determination on the disposition of the carcass (Kaneene *et al*., 2006; USDA/FSIS 2009a). If the lesion is histocomptible with Mycobacteriosis, then PCR and culture are then performed at NVSL (Kaneene *et al*., 2006; USDA/APHIS/VS, 2009c). Polymerase chain reaction (PCR) results indicating whether the tissues are *Mycobacterium tuberculosis* (MTB) complex can be available as rapidly as 24-48 hours and a definitive diagnosis of *M. bovis* can be made by culture (bacteriological isolation) within four to eight weeks (Thomson, 2006).

An optimal submission rate for adult cattle for locating the final sources of bovine TB in the U.S. was statistically determined to be at least one lesion per 2,000 adult cattle inspected at slaughter establishments inspecting primarily adult cattle (breeding animals) (Gilsdorf *et al*., 2006a). The rate was established in 1969 according to Wagner (1988) and in 1972 according to USDA/APHIS/VS (1990) and Essey and Koller (1994). Neither USDA literature nor published literature has cited an optimal submission rate for fed cattle. Without an optimal submission rate for fed cattle, there is concern that an inadequate number of bovine TB lesions from fed cattle will be tested and these lesions will not be included in the search for the final sources of the disease.

Epidemiological trace-back investigation

Upon histo-pathologic diagnosis of compatibility for Mycobateriosis (a presumptive diagnosis of bovine TB), the USDA-APHIS initiates an epidemiological trace-back investigation to attempt to locate (USDA/AHPIS, 2005a & 2005c; Kaneene *et al*., 2006):

      1) the herd (index farm) of origin of the bovine TB lesioned animal (as well as all

      locations where the animal was prior to slaughter: feedlot, market, farm, etc.);

      2) the herd(s) from which the infection came; and

3) any additional exposed animals or herds.

The investigation begins with the presumptive diagnosis from histopathology (USDA/APHIS/VS, 2005c) because a definitive diagnosis dependent on culture isolation and identification of *M. bovis* can take up to 4-8 weeks (Thomson, 2006). Despite the fact that PCR has helped decrease the time required to ascertain whether the acid fast bacteria observed in the tissue was *M. bovis*, (Thomson, 2006), investigations typically begin with the results of histopathology and involve an epidemiological field investigation to determine the herd of origin of the lesioned animal.

The field investigation begins with notification of the bovine TB case to State and Federal personnel, such as the Area Veterinarian in Charge, VS Regional Tuberculosis Epidemiologist, Designate Tuberculosis Epidemiologist (DTE) and State animal health officials. State or Federal animal health officials review and reconcile information pertaining to the lesioned animal that was collected during the slaughter process (*e.g.* slaughter plant kill sheets, the consignors for the slaughter lot where the animal resided, the VS Form 6-35 completed during post mortem exam, any available animal identification). If there is enough information available at slaughter, the investigation proceeds and all available receipts and records (including interstate certificates of veterinary inspection (ICVI) detailing the infected animal's movements between states, various owners, livestock markets, and/or feedlots are reviewed. Bovine TB found during slaughter inspection is considered to have originated in the State where the animal was slaughtered or where the disease was disclosed, unless successful trace-back procedures identify another State as the original source (USDA/AHPIS, 2005a). The investigation of bovine TB cases must be completed within 90 calendar days of laboratory notification of positive PCR or bacteriological isolation of *M. bovis*; however, the timeframes can be extended in certain situations (USDA/AHPIS, 2005a).

If *M. bovis* is confirmed in a (index) herd (or herd of origin), the subsequent investigation may include identification of all contact herds:

- adjacent (neighboring) and surrounding herd(s),
- trace-ins to find the source of infection, and

- trace-outs through registered sales and livestock auction markets to find other exposed animals and herds (USDA/APHIS/VS, 2006a).

Herds identified as the source(s) of slaughter trace-back case investigations are notified and placed under quarantine within 15 days of receiving notification and a herd test of all eligible livestock is scheduled (USDA/AHPIS, 2005a). The caudal fold tuberculin (CFT) test is the primary test used for initial screening for bovine TB in cattle herds suspect of bovine TB (USDA/APHIS/VS, 2009c). Responders to the CFT test may be further classified with the comparative cervical tuberculin (CCT) test or bovine interferon gamma assay (USDA/APHIS, 2005a). Testing and animal classification decisions are made by the DTE, in consultation with the Regional Tuberculosis Epidemiologist. Herds with confirmed *M. bovis* infection (by evidence with histopathology, PCR assay, bacterial isolation or detection, testing data or epidemiologic evidence, such as contact with known sources of infection according to CFR, Title 9, Chapter 3, Section 77, Part 2) are labeled "affected herds" by the USDA/APHIS (USDA/APHIS, 2005a; CFR 2010c). Affected herds remain under quarantine until depopulation or completion of an individual herd plan subject to test-and-remove protocols (USDA/APHIS, 2005a).

In order to identify all potential sources of infection that might have infected the cattle in the affected herd, investigators may trace cattle that were added (purchased, inherited, gifted, etc) to the herd over the previous 5 years; however, the number of years they review can vary by situation (USDA/APHIS/VS, 2011g). These secondary investigations, known as "6-4A trace-ins," can involve large numbers of cattle from multiple premises. The source herd(s) (of the reactor(s) that were disclosed in the affected herd) are given the CFT test procedure; responding animals may be classified as reactors, or if classified as suspects, may be retested by the CCT test or bovine interferon gamma assay (USDA/APHIS, 2005a).

In order to determine if other animals/herds were exposed to *M. bovis* infection, investigators trace the movement of cattle that left the affected herd or were exposed to the affected herd during the previous five years (USDA/APHIS/VS, 2011g). These investigations are referred to as '6-4B trace-outs'. There can be multiple trace-outs involving large numbers of cattle who have been sold or moved to multiple premises.

Trace-outs are typically conducted via records from registered sales and livestock auction markets. Herds containing known bovine TB exposed animal(s) are placed under quarantine until the bovine TB status of the exposed animal(s) has been determined by postmortem examination or by at least one negative Cervical Tuberculin (CT) test, and the remainder of the test eligible animals in the herd are determined to be negative following an official CFT test (USDA/APHIS, 2005a). When trace-in and trace-out investigations yield affected herd(s), the herds are depopulated or individual herd plans are developed with test-and-remove protocols.

After bovine TB infected ("affected") herds are found and addressed, or all leads on a trace-back investigation have been exhausted, the investigation is summarized in a case closing report along with the primary reason for closing the investigation. All case work documentation, including the post mortem form (VS Form 6-35), all histopathology, PCR and culture laboratory results, results of CFT, CT, CCT and gamma interferon testing (on the herd of origin, 6-4A trace-ins and 6-4B trace-outs), investigation notes and emails and the case closing report are compiled in a case file. This information is retained in physical case files and electronic files at state and federal offices. To track and summarize each investigation occurring across the nation, USDA/APHIS/VS bovine TB program personnel maintain a summary spreadsheet of all laboratory cases that were compatible for Mycobacteriosis by histopathology. For each case, the spreadsheet includes the fiscal year, National Veterinary Services Laboratories (NVSL) accession number, state primary (the state where the slaughter plant was located or the State that has been identified as the original source if different than the slaughter plant state), state secondary (becomes the slaughter plant state, if cattle came from a state outside the slaughter plant state), available animal identification, country or (Mexican) state of origin, slaughter date, owner (most recent prior to slaughter), age, sex, laboratory results such as histo-compatible for Mycobacteriosis, PCR and culture results, investigation status and comments, whether the case is closed, case closure date, days from slaughter to closure, eligible for award, award comments, award amount, inspector state, slaughter establishment and name of inspector (USDA/APHIS/VS, 2009c).

1.2.3 Bovine TB screening and diagnostic tests

One or a combination of tests can be used for screening and diagnosis of bovine TB: 1) the cervical

tuberculin (CT) test, 2) the caudal fold tuberculin (CFT) test, 3) the comparative cervical tuberculin (CCT)

test, and 4) the bovine interferon gamma assay. The CT and CFT test are also known as single intradermal

tuberculin (SIT) tests, and the CCT is also known as a single intradermal comparative cervical tuberculin

(SICCT) test. None of the tests mentioned above are perfect in detecting bovine TB; however, the

tuberculin tests have been used for the diagnosis of bovine TB in cattle for more than 100 years

(Monaghan, *et al.*, 1994) and, when testing has been combined with the culling of reactors, controlling

bovine TB in cattle has been remarkably successful in many countries throughout the world (Nolan

&Wilesmith, 1994). As mentioned by Michel *et al.* (2010) "The breakthrough in the eradication of bovine

and zoonotic tuberculosis in developed countries was achieved through mandated tuberculin testing of

livestock and removal of positive reactors and compulsory pasteurization of milk." Additionally,

tuberculin testing has proven over time to be more reliable than post mortem results as a means of detecting

infection (Anderson, 1959b). The advantage of tuberculin testing over slaughter surveillance has been the

ability to detect infected animals before they reach advanced stages of disease.

1.2.4 Bovine TB screening and diagnostic tests used in the U.S.

Since the 1960's, the tuberculin skin test has not been the principle means by which infected herds are

identified in the U.S. under the bovine TB control and eradication program; instead, it is a secondary

method used primarily in response to finding a bovine TB lesion at slaughter. All four tests, the CT, CFT,

CCT and bovine interferon gamma assay, are official tuberculosis tests for cattle in the U.S.

(USDA/APHIS, 2005a). The CFT test is most frequently used test in the U.S. as it is the official

presumptive diagnostic test for routine use in individual cattle and herds where the bovine TB status of the

animal is unknown. The CCT test and bovine interferon gamma assay are supplemental (or ancillary)

diagnostic tests. The CCT is an official test for retesting cattle suspects to the CFT and the bovine

interferon gamma assay may be only be used with appropriate approval as an official supplemental

diagnostic test. The CT test can be used as a primary diagnostic test, recommended for use in herds

affected with bovine TB and is required as the initial test for testing exposed cattle from such herds. In lieu

of the CT test, the CFT test is also a primary diagnostic test for cattle in herds affected with bovine TB (USDA/APHIS, 2005a). The interferon-gamma test is used in cattle herds in the U.S. as a replacement for the CCT test of retesting CFT test suspects, in parallel with the CFT test or CT in affected herds, and rarely, in parallel testing with the CCT test (USDA/APHIS, 2005a; Prionics, 2011).

In 1960, the USDA was annually tuberculin testing enough cattle to approximately equal 10 percent of the cattle population; as a result of the change in emphasis in control methods, area testing declined by approximately 63 percent from 1965 to 1970 (Ranney, 1970). In 1970, approximately 20 percent of the cattle population was surveyed for bovine TB annually through the combination of tuberculin testing and meat inspection (Ranney, 1970). In 1988, routine herd testing detected relatively few herds each year, with detection of bovine TB now in the hands of meat inspection personnel (Meyer, 1988). In 2001, Dr. Meyer with USDA:APHIS:VS, explained that surveillance by skin testing, including periodic bovine TB testing of cattle on farms throughout the U.S., played a relatively minor role in detecting bovine TB-infected herds since fewer than one million tuberculin tests are conducted annually (USDA/APHIS/VS, 2001a).

In comparison to earlier in the life of the program, relatively few herds are now tested for herd accreditation purposes and few cattle are tuberculin tested. In FY 2007, of the 571,979 CFT tests performed in Accredited-free states, only 10.9% of these were for herd accreditation; the remaining 33.5% of the tests were for private sale, 19.0% for other reasons, 13.0% for milk ordinances, 9.3% for show, 5.7% for movement, 6.2% for import, 1.4% for export and 0.8% for area (USDA/APHIS/VS, 2009c). For FY 2007, in the 49 Accredited-free States, 62,627 individual CFT tests were performed on cattle for herd accreditation (or re-accreditation) and 557 total herds were tested for herd accreditation (or re-accreditation) (USDA/APHIS/VS, 2009c). As of September 2008, only an estimated (more than) one million head of cattle were tested annually (USDA/APHIS/VS, 2008c) of the estimated 96 million head of cattle in the U.S. (as of Jan 1, 2008 the cattle inventory was 96.0 million head) (USDA/Economic Research Service [ERS], 2011). Table 1 shows the number of U.S. cattle, caudal fold tests (CFT) performed on cattle and bison and the number of responders (percentage) for 2006 to 2010. For FY 2010, 1,275,815 CFT tests of cattle and bison were reported with 18,217 responders or 1.4% (USAHA, 2010).

Table 1: Number of U.S. cattle, caudal fold tests and responders for cattle and bison, 2006-2010

	2006	2007	2008	2009	2010
Cattle inventory[a]	96,300,00	96,600,000	96,000,000	94,500,000	93,900,000
Total tests[b]	1,106,669	1,135,725	1,366,186	1,171,854[c]	1,275,815
Responders[b]	10,916	16,336	20,229	19,164[c]	18,217
Percentage, overall[b]	1.0%	1.4%	1.5%	1.6%[c]	1.4%
Percentage, by state (≥ 300 tests)[b]	0.0 – 3.3%	0.0 – 3.2%	0.0 – 3.9%	0.0 – 4.5%[c]	0.1 - 6.8%

[a] As of January 1 of the respective year (Source: USDA/ERS, 2011a)
[b] For fiscal year October to September (Sources: USDA/APHIS/VS, 2010c; USAHA, 2010)
[c] As of September 21, 2009, 46 States and Puerto Rico (Source: USDA/APHIS/VS, 2010c)

Today, cattle are tuberculin tested for the following reasons (Ranney, 1970, USDA/APHIS/VS, 2008c and 2009c):

    1) suspect of having bovine TB,

    2) part of an epidemiological investigation, as preliminary screening to test a herd suspected of

    bovine TB, when,

        (a) a bovine TB lesioned animal is traced from slaughter back to a herd of origin or to an

        exposed herd, or

        (b) for selective area testing in high risk areas (high risk epidemiological testing[b])

    2) preparing for interstate movement (from areas where bovine TB is known to occur), import or

    export,

    3) required for participation in a show or exhibition,

    4) required for a change in ownership (private sale),

    6) part of surveillance activities at slaughter

    7) milk ordinance, and

    8) herd accreditation.

[b] *Note: According to the Bovine TB Eradication Uniform Methods and Rules, section K, high risk*

epidemiological testing consists of (USDA/APHIS, 2005a):

    1) post-quarantine annual retests of old infected herds,

    2) post-depopulation annual retest of repopulated herds,

    3) herds having lesions suggestive of bovine TB but laboratory results were inconclusive,

4) herds receiving known exposed animals,

5) herds identified as a possible source of infection for another herd,

6) herds adjacent to or in contact with an infected herd, and

7) herds located in historically "high-risk" areas (i.e. El Paso milkshed, Texas).

In order to classify reactions to the tuberculin test, the U.S. uses the following categories (USDA/APHIS, 2005a):

A) responder - any cattle or bison officially skin tested for tuberculosis that has a visible or palpable response at the site of tuberculin injection,

B) reactor - any cattle/bison that shows a response to an official tuberculosis test,

C) suspect - any cattle/bison that show a response to the CFT test and are not classified as reactor, or any cattle that have been classified as suspects by CCT tests and the bovine interferon gamma assay, and

D) negative - any cattle/bison that show no response to an official tuberculosis test or classified negative on the bovine interferon gamma assay if conducted.

Caudal fold tuberculin (CFT) test

The intradermal tuberculin test was adopted as the official test in 1922 (Francis, 1947b). To perform the CFT test as prescribed by the U.S. 2005 UM & R, an intradermal injection of 0.1 ml of USDA bovine purified protein derivative (PPD) tuberculin (1 mg/ml PPD) must be administered into either side of the caudal fold, with reading by visual observation and palpation 72 hours (plus or minus 6 hours) following injection (USDA/APHIS, 2005a). A positive reaction is recorded if there is any discoloration or increase in skin thickness with or without clinical signs (USDA/APHIS, 2005a; USDA/APHIS/VS, 2006a). Animals responding to the CFT test are subject to a CCT or interferon gamma test. The CCT must be administered within 10 days of or no earlier than 60 days after the CFT tuberculin injection date. In the U.S., all responses to the CFT test are classified as "suspect", unless the reactor classification is indicated by the professional judgment of the DTE. If the animal is a "suspect" for CFT, additional testing will be conducted using CCT or GI (USDA/APHIS, 2005a).

Cervical tuberculin (CT) test

Similar to the CFT, the cervical tuberculin (CT) test, requires an intradermal injection of 0.1 ml of USDA bovine cervical PPD tuberculin (2 mg/ml PPD) in the cervical region with reading by visual observation and palpation 72 hours (plus or minus 6 hours) following injection (USDA/APHIS, 2005a). A positive reaction is recorded if there is any discoloration or increase in skin thickness with or without clinical signs (USDA/APHIS, 2005a; USDA/APHIS/VS, 2006a). The results of a CT test can only be classified as reactor or negative (USDA/APHIS, 2005a).

Comparative cervical tuberculin (CCT) test

The comparative cervical tuberculin (CCT) test was adopted in the U.S. in 1973 in attempt to better differentiate *M. bovis* caused tuberculin responses from those caused by non-specific agents (Meyer, 1988). As mentioned above, the comparative cervical test (and gamma interferon test) are primarily used in the U.S. as confirmatory tests (in series), only animals that respond positively (suspects or reactors) to the CFT test are given the CCT (bovine interferon gamma assay) to confirm diagnosis of *M. bovis* infection (Kaneene *et al*., 2006; CFSPH, 2007; USDA/APHIS/VS, 2011g). The CCT injection must occur either within 10 days following the CFT injection or more than 60 days following the CFT injection (USDA/APHIS, 2005a). The CCT test, requires an intradermal injection of biologically balanced USDA bovine PPD tuberculin (0.1 ml) and avian PPD tuberculin (0.1 ml) at separate sites in the midcervical area to determine the probable presence of *M. bovis* by comparing the response to the tuberculins at 72 hours (plus or minus 6 hours) following injection (USDA/APHIS, 2005a). The test result is a comparison of the increase in skin thickness resulting from the two injections. A positive result is recorded when the bovine reaction is more than 4 mm greater than the avian reaction or local clinical signs of oedema, exudation, necrosis or pain are present at the bovine site. An inconclusive result is recorded when the bovine reaction is from 1-4 mm greater than the avian reaction and there are no clinical signs at the bovine site. A negative result is recorded when a negative bovine reaction, or positive or inconclusive bovine reaction which is equal to or less than a positive or inconclusive avian reaction is recorded in absence of local clinical signs. Animals inconclusive to the CCT can be re-tested after a minimum or 42 days (Monaghan *et al.*, 1994). In

the U.S. responses to the CCT test are recorded and plotted on a scatter gram and classified, as negative, suspect, or reactor, according to the zone in which the animal is plotted. Cattle plotting in the suspect zone on two successive CCT tests are classified as a "reactor". If an animal is a "suspect" (inconclusive) for CCT, they may be retested by CCT 60 days later or are sent to directly to slaughter (Kaneene *et al.*, 2006).

Bovine interferon gamma assay

The bovine interferon (IFN) gamma (γ) assay, marketed as Bovigam[TM] by Prionics®, is a blood-based assay of cell mediated immunity that has been available as an official test in the program since 2005 (USDA/APHIS/VS, 2008d; Prionics, 2011). The *in vitro* laboratory test for the diagnosis of bovine TB involves culturing (whole) blood with bovine and avian tuberculin (Tuberculin Purified Protein Derivatives (PPD) antigens). When the tuberculins are presented to lymphocytes, the assay system (monoclonal antibody-based sandwich enzyme immunoassay) detects and quantifies the production of the cytokine interferon gamma from stimulated T cells (against the tuberculins) (Rothel *et al.*, 1990; Prionics, 2011). Lymphocytes from uninfected cattle do not produce IFN-γ to tuberculin PPD antigens and hence IFN-γ detection correlates with infection (Prionics, 2011).

The IFN-γ test has several advantages over the tuberculin skin test, including the fact that it can differentiate between infected and vaccinated individuals, only requires that the animals be handled once, can identify animals at an earlier stage of infection and can be conducted in a controlled laboratory setting (providing more objective, consistent and reliable results) (Pollock *et al.*, 2005; USAHA, 2009b; Michel *et al.*, 2010; USDA/APHIS/VS, 2010c). Experimental infections in cattle have shown that the IFN- γ test can detect infection as early as 14 days post-infection, which is earlier than what has been reported with the SIT (Buddle *et al.*, 1995). Since the IFN- γ test can identify animals at earlier stage of infection than the tuberculin skin test does, it can detect infected cattle that escape detection by the SIT test when used in series (Neill *et al.*, 1994, Monaghan *et al.*, 1997; Wood & Jones, 2001; Vordermeier & Goodchild, 2003, unpublished data; Pollock *et al.*, 2005). Animals positive on a single IFN-γ test are classified as "suspect" unless the DTE or Regional Tuberculosis Epidemiologist determines that a "reactor" classification is

justified. Animals positive on two successive IFN-γ tests are classified as "reactor" (USDA/APHIS, 2005a).

Protocols for a herd determined to be infected ("affected")

An "affected" herd is classified if *M. bovis* infection has been confirmed within a herd based on the results of testing and post mortem examination of responders. The first consideration for action is depopulation of the entire quarantined herd (USDA/APHIS, 2005a). Any livestock, for which Federal indemnity may be paid because of bovine TB, must be destroyed and the carcass disposal completed within 15 days after the date of appraisal; the Veterinarian in Charge may extend the time limit to 30 days and the Administrator may extend the limit beyond 30 days upon request in specific cases according to CFR, Title 9, Chapter 1, Section 50, Part 7 (CFR, 2010). If depopulation cannot be accomplished, an individual herd plan will be devised and the herd will remain under quarantine until all requirements of the approved plan have been completed. Beginning in 2005, the testing requirements for an individual herd plan included eight required herd tests for quarantine release, with an additional two negative annual herd tests after quarantine release (USDA/APHIS, 2005a). However, this standard was changed in 2010 to fewer tests to achieve quarantine release, combined with 3 to 5 assurance tests during a several year period after quarantine release (USAHA, 2010 and 2011).

1.3 Impediments to the eradication of TB in the U.S.

The U.S. national bovine TB eradication program has proved highly effective and has been sustained since 1917. Despite persistent efforts and the low prevalence of bovine TB in the U.S., the goal of eradication has remained elusive as animal health officials continue to detect bovine TB sporadically in U.S. livestock herds (USDA/APHIS/VS, 2009b). The extensive initial successes with bovine TB seen in the U.S. during the "Achievement Days" (Anderson, 1959a) were achieved at a time when herds where smaller, the intensity of production was lower than it is currently and (as far as we know) before the involvement of a wildlife reservoir of *M. bovis* (Ranney, 1970; Whipple & Palmer, 2000). Over time, various impediments to eradicate bovine TB from the national herd in the U.S. had been described. Anderson (1959a) described

a number of perceptions that were widely accepted in 1959 that may have served as socio-economic impediments for the bovine TB eradication effort:

- an irreducible minimum had been reached with bovine TB and the U.S. beef and dairy industries would have to live with a certain amount of bovine TB;

- control measures were adequate;

- bovine TB was a vanishing disease and no longer an economic problem;

- bovine TB as a public health hazard was non-existent; and

- the bovine TB victory had been won, the need for vigilance was gone, pressures for vigorous action withdrawn and to continue pushing the eradication effort was a waste of time and money

In 2009, the USDA formally described the eight items below as major challenges for the eradication of bovine TB in U.S. national cattle herd (USDA/APHIS/VS, 2009b and 2009d):

1) infected cattle imported from other countries,

2) infected wildlife as a reservoir,

3) changes in the dairy and beef industries,

4) outdated regulations,

5) antiquated approaches to disease control,

6) flat or decreasing federal budgets,

7) limitations of available diagnostic tests, and

8) inability to trace some infected animals identified at slaughter to a herd, also described by the USDA as the absence of a fully implemented national ID system negatively impacts the ability to identify affected herds.

1.3.1 Imported infected cattle

The U.S.'s primary cattle trading partners are Canada and Mexico (USDA/ERS, 2009). For 2002-2010, U.S. cattle imports from all sources averaged 2.09 million head annually, with an average of 0.989 million head annually from Canada (USDA/ERS, 2011). For 1989 to 2010, U.S. cattle imports from Mexico averaged over 1 million head annually (Peel *et al.*, 2011). Recent trends show that the number of cattle

imported from Canada is increasing while the number imported from Mexico is decreasing

(USDA/APHIS/VS, 2011g). Canada and Mexico have established national bovine TB eradication

programs, similar to the one used in the U.S.; Canada in 1923 and Mexico in 1993 (Essey & Koller, 1994;

Whipple & Palmer, 2000).

Canada's animal health officials consider all of their provinces as bovine TB-free; consequently, Canadian

cattle are considered to present a low risk of bovine TB introduction to U.S. cattle (USDA/APHIS/VS,

2011g). In 1997, the USDA recognized all Canadian cattle herds that were not under quarantine as bovine

TB free for the purpose of import requirements, freeing Canadian exporters from the time and expense of

testing and holding cattle for 72 hours before shipment to the U.S. (Lee, 2004). In August 2004, due the

discovery of bovine TB in a number of cattle herds in the vicinity of Riding Mountain National Park

(RMNP), APHIS reinstated testing requirements for imported cattle having resided in the province of

Manitoba (Lee, 2004). The Province of Manitoba has remains classified as modified accredited advanced

(MAA) for their bovine TB status by the USDA for import purposes (NCIE, 2007). Since 2007, the

import-export laws with Canada have been the following:

- No tests are required for bovines that are certified by Canadian Food Inspection Agency (CFIA) as
  continuously residing in a bovine TB-free province.

- For animals from Manitoba, no test is required if

  a)   the bovines are moved directly to slaughter,

  b)   the bovines are sexually intact heifers, steers or spayed heifers moved to a feedlot, or

  c)   the bovines are from an accredited herd and are accompanied by a certificate which states
       that the accredited herd completed the necessary tests for accredited status with negative
       results within 1 year prior to the date of movement.

- For Manitoba cattle, not meeting any of the three scenarios above, an official tuberculin test is
  required within 60 days prior to the date of movement (NCIE, 2007).

Due to the prevalence of bovine TB in Mexico, Mexican cattle are considered to present a high risk of

bovine TB introduction to U.S. cattle. Since the late 1990s, the prevalence of bovine TB affected herds in

many Mexican States has substantially declined (USDA/APHIS/VS, 2009d). APHIS has recognized

equivalent bovine TB status of some Mexican States and zones consistent with the current U.S. bovine TB classification scheme: Modified Accredited Advanced (MAA), Modified Accredited (MA), Accreditation Preparatory (AP) and non-accredited (USDA/APHIS/VS, 2011g); however, no Mexican states are currently Accredited-free (AF). Since 2001 (when an interim rule was published in the U.S. Federal Register (66 FR 20187-20190, Docket No. 00-102-1) and was later added to Part 93 (Section 404 and 406) of Title 9 of the Code of Federal Regulations), the following rules have applied to any imported cattle, including imported Mexican cattle:

- any imported cattle, not going directly to slaughter, must have a health certificate attesting that the cattle have tested negative for bovine TB,
- no cattle from a herd with evidence of bovine TB or suspected with bovine TB are allowed entry into the U.S. until the herd achieves accredited status (by way of a negative whole herd test for bovine TB within 1 year prior to the date of exportation to the U.S. and the animals each test negative to an additional official tuberculin test conducted within 60 days prior to the date of exportation to the U.S.), and
- the importation of Holstein steers, spayed heifers, Holstein cross steers and Holstein cross spayed heifers from Mexico is prohibited (CFR,2010g and 2010h).
- all cattle entering from bovine TB Mexican zones must be branded with an "M" unless the animals are shipped directly to slaughter (USDA/APHIS/VS, 2011g).

Epidemiological investigations conducted by VS and the States, have indicated that many bovine TB infected cattle detected at slaughter were imported and originated from Mexico (Essey & Davis, 1997; USDA/APHIS/FSIS internal document by Reed, 1999; Whipple & Palmer, 2000; USDA/APHIS/VS, 2009d). Despite the testing requirements for Mexican cattle prior to importation, there have been many instances in which cattle legally imported from Mexico were later found to be positive for bovine TB at slaughter, likely due to the sensitivity of the original screening test (USDA/APHIS/VS, 2011g). Mexican-origin cattle were determined to have played a role in the source of *M. bovis* in California and a predominant role in the source of *M. bovis* in New Mexico (USDA/APHIS/VS, 2011g). Once imported, there are no further regulations on imported cattle (Naugle, 2011). As such, the importation of bovine TB,

particularly from Mexican-origin cattle, has been and continues to be a risk factor for U.S. cattle (USDA/APHIS/VS, 2011g).

A large percentage of the imported cattle from Mexico are young steers and heifers, commonly referred to as fed or feeder cattle, intended for stocker operations (backgrounding) and eventual finishing in U.S. feedlots, and a very small percentage are event (competition) cattle or rodeo cattle (USDA/ERS, 2009; Peel *et al*., 2011; USDA/APHIS/VS, 2011g). The length of time that Mexican cattle are in the U.S., capable of spreading disease if infected, is important epidemiologically. After being imported, Mexican origin fed cattle typically are on pasture for 6 to 8 months prior to entering feedlots from which they eventually move directly to slaughter (Essey & Koller, 1994). APHIS' Veterinary Services' found that Mexican origin bovine TB infected fed cattle detected at slaughter (in the U.S.) resided in the U.S. a median of 10 months (range 4.6 to 16 months, n=22) before they were sent to slaughter (USDA/APHIS/VS, 2007a).

If Mexican cattle are exposed to or commingled with U.S. breeding cattle (cows, bulls, and replacement heifers) (for example, on pasture, in feedlots, at fence lines, or in other circumstances), then bovine TB can be transmitted to the U.S. domestic herd. Transmission may occur through direct contact or indirectly through contaminated feed and water troughs or other fomites. When management practices in the U.S. have presented opportunities for Mexican feeder (or roping) cattle to be commingled with domestic cattle, there has been opportunity for exposure and transmission of bovine TB (USDA/APHIS/VS, 2011g). Reviews of bovine TB outbreaks at dairy operations showed that many of them used offsite heifer raising facilities, raising concerns about the commingling of animals on these operations and the potential exposure to Mexican cattle, which could result in the transmission of multiple diseases, including bovine TB (USAHA, 2010). According to the NAHMS Dairy study in 2007, offsite heifer-raising operations are becoming more common and are now used by about 1 of 10 dairy operations; almost one-half of operations with 500 or more cows raised at least some heifers offsite (USAHA, 2010).

Mexican cattle used in events and competition (rodeo/roping cattle) may present a higher risk to domestic cattle, than imported infected feeder cattle destined for slaughter, given their longevity and frequent and

extensive movement around the country before slaughter. In the off season, imported rodeo cattle are often

moved to pasture where they may commingle with domestic cattle populations (USDA/APHIS/VS, 2011g).

Mexican cattle that illegally enter the U.S. also pose a risk to the U.S cattle industry. The illegal entry of

cattle may be intentional by smugglers or unintentional through areas along the border where fencing is

limited or damaged (USDA/APHIS/VS, 2011g). Using bovine TB cases in cattle of Mexican origin and

Mexican cattle import records, the incidence of bovine TB cattle imported from Mexico into the U.S. per

100,000 has been decreasing. From 1995 through 1997, the rate ranged from 7.3 to 18.7 infected cattle per

100,000 imports annually and from 1998 to 2009, the rate ranged from 0.2 to 5.4 (USDA/APHIS/VS,

2007a, 2008d, and 2009a). Since 2003, the number of Mexican origin bovine TB cases identified at

slaughter has decreased consistent with the decrease in the number of cattle entering the U.S.

(USDA/APHIS/VS, 2011g). Despite pre-import testing requirements and a sustained decrease in the

incidence of bovine TB cattle imported from Mexico, imported bovine TB infected cattle from Mexico

continue to be detected at slaughter and a remain a concern for the health of the U.S. (domestic) herd

(USDA/APHIS/VS, 2008d and 2009d; USAHA, 2009b).

1.3.2 Wildlife as a bovine TB reservoir

Wildlife infected with *M. bovis* can serve as a source of infection and transmit the disease to domestic

livestock. On the island of Molokai in Hawaii, *M. bovis* was isolated from free-ranging axis deer in 1970

and feral swine in 1980. It was determined that the organism had become established in the feral swine

population, thus they were recognized as the first wildlife reservoir of *M. bovis* in the U.S. (Whipple &

Palmer, 2000). In 1975, the first bovine TB wild cervid was detected in Michigan but was believed to be a

spillover from an infected cattle herd (Schmitt *et al*., 1997) and in 1994 a second animal was found

(Corner, 2006). In 1995, the first report of self-sustaining bovine TB in wild, free-ranging U.S. cervids (an

endemic focus bovine TB infection) was found in free-ranging white-tailed deer in the northeastern Lower

Peninsula of Michigan. From 1996 through 2003, there were 32 outbreaks of bovine TB in cattle in the

area (Corner, 2006). Guidelines for eradication of bovine TB from captive members of the family Cervidae

were added to the UM&Rs in 1994 (USDA/APHIS, 1999). From 1991 to 2004, 41 infected captive cervid

herds were discovered in the U.S. (Gilsdorf *et al*., 2006a). Bovine TB in wildlife is the primary reason

infected ("affected") cattle and captive cervid herds continue to be found in Michigan. In Minnesota, a bovine TB infected beef cattle herd was confirmed in 2005 in Roseau County (USDA/APHIS/VS, 2011g). Since 2005, 26 bovine TB positive wild deer have been found, all within a 10-mile radius of the first bovine TB confirmed herd in Roseau County (Carstensen *et al*., 2011 and State of Minnesota, 2011) and by 2009, 12 beef herds in Minnesota were identified as bovine TB positive (USDA/APHIS/VS, 2011g and Carstensen *et al*., 2011). In 2009, an unprecedented number (seven) of captive cervid herds were detected in the U.S. (USDA/APHIS/VS, 2009a). As of 2011, the U.S. has testing requirements for accreditation and interstate movements of captive cervids and some passive surveillance through slaughter channels; however, there is no official bovine TB slaughter surveillance program for cervids (USDA/APHIS/VS, 2011g).

Michigan and Minnesota each had only one strain of *M. bovis* in their infected cattle, an indication of a potential point source of introduction followed by local area spread. The same strains, were also identified in the wildlife of each respective State, indicating cattle contact with infected white-tailed deer (especially via contact with feed contaminated by deer) is an important risk factor for the introduction and spread of bovine TB (USDA/APHIS/VS, 2011g). Michigan and Minnesota used the same basic control strategies but the management of the disease in Minnesota started earlier, was more aggressive and was implemented more rapidly (Carstensen *et al*., 2011). Consequently, available evidence has suggested that white-tailed deer in Michigan are now self-sustaining maintenance hosts, whereas in Minnesota they are still a spillover host of bovine TB (Schmitt *et al*., 1997). In Michigan, since 1995, the prevalence of bovine TB in free-ranging deer has decreased from 4.9% to 1.8% in the ~1500 km^2 core outbreak area. The reduction was facilitated by liberalized hunting and restrictions on baiting and feeding; however, there has been little support from hunters, farmers or the general public for more aggressive population reduction measures, such as culling. Additionally, compliance with baiting and feeding restrictions has been variable and often problematic (Carstensen *et al*., 2011). In contrast, in Minnesota from 2005 to 2008, disease prevalence in deer has decreased from 0.4% (SE=0.2%) to <0.1% and remained confined to a small (<425 km^2) geographic area. The reduction was facilitated by liberalized hunting and targeted culling by ground sharpshooting and aerial gunning, combined with a prohibition on baiting and recreational feeding.

Support from cattle producers, deer hunters, politicians and the general public made the implementation of these aggressive strategies by state and federal authorities possible (Carstensen *et al*., 2011). As a result of their different responses, Michigan and Minnesota are in widely different situations today; Michigan, with established disease in their white-tailed deer population, is likely to face a prolonged battle with the disease. O'Reilly and Daborn (1995) indicated that bovine TB is a readily controllable disease when there is no wildlife reservoir of *M. bovis* infection; however, when there is transmission of infection from endemically infected wildlife populations to cattle, eradication is not feasible and control measures must be applied indefinitely.

In Michigan, the high prevalence of *M. bovis* infection in free-ranging deer has been proposed to be linked to the changed land use, that has increased the likelihood of interaction with cattle, and changed management and supplemental feeding of the wild deer population that lead to dramatically increased numbers and changed behavior (O'Brien *et al*., 2002; Schmitt *et al*., 2002, Cutler *et al*., 2010). The transmission process between wild deer and cattle is unclear since studies of cattle and deer behavior on pasture have revealed that direct interactions occur rarely (DeLiberto *et al*., 2002). Nevertheless, transmission is still speculated include aerosol, ingestion, involvement of a third animal species and/or by another uncommon and less obvious route (Corner, 2006). If cattle and captive cervids reside on the same premise, transmission can readily occur with the inhalation of aerosols, face-to-face contact, physical contact with feed, water or minerals that have been contaminated by saliva or other discharges (USDA/APHIS/VS, 2011g). Eradicating bovine TB in free-ranging wildlife is complex and requires a great deal of cooperation between state and federal livestock and wildlife officials and the livestock owners (USAHA, 2009b). The identification of bovine TB in wildlife has impacted the direction and success of the U.S. bovine TB control and eradication program for the last decade and will continue to be a significant challenge in the future (USDA/APHIS/VS, 2009d).

1.3.3 Changes in the U.S. dairy and beef industries

Changes in the U.S. dairy and beef industries, in regards to the size of herds, management practices and increased specialization of producers, have been associated with the transmission of bovine TB among

cattle. Historically, dairy herds have been more likely to be infected with bovine TB and more extensively infected than beef herds; the facility layout, activities conducted, and stability of dairy operations offer a greater chance for exposure to bovine TB than beef operations which are more open and transient in nature (Morris *et al*., 1994; Schoenbaum & Meyer, 1995; USDA/APHIS/VS, 2008c, USDA/APHIS/VS, 2011g). The dominant concept in bovine TB control has been, and to a large degree still is, the importance of close contact (Morris *et al*., 1994). Since dairy cows, spending more time in enclosed areas or in crowded conditions, they are at higher risk of exposure than beef cattle (USDA/APHIS/VS, 2008c). Evidence has suggested that bovine TB transmission is more efficient when animals are housed close together; when heifers were pastured with heavily infected cows, the incidence remained low until they enter the cowshed (Morris *et al.*, 1994; Schoenbaum & Meyer, 1995).

Today's cattle industries feature fewer herds of increased size (USDA/APHIS/VS, 2009d). From 1990 to 2010, the number of all cattle operations in the U.S. has fallen 28% and beef cow operations have declined by 21%; however, the average number of cattle per beef cow operation has increased by 36%, to roughly 100 heard for all cattle operations, and in 2009, operations with 500 head or more accounted for 47.7% of the total cattle inventory, compared to 38.0% in 1999 (USDA/APHIS, 2011h). In regard to the dairy industry, milk cow operations decreased from 97,460 in 2001 to 65,000 in 2009. Additionally, between 2001 and 2009 the number of operations with 500 or more head increased from 2,795 to 3,350 (by 20%), operations with 2,000 or more head increased from 325 to 740 (128%) and operations with fewer than 500 head decreased from 94,665 to 61,650 (a decline of 35%)(USDA/AHPIS, 2011h). Officials have concluded that it is more difficult to eliminate bovine TB from today's large dairies than the smaller dairies of the past based on experiences with test-and-removal in dairies in the El Paso milk shed (in TX and NM) (Schoenbaum & Meyer, 1995). Multiple factors can influence the transmission of bovine TB within a large herd: the number of susceptible animals, the number of infected animals, the number of animals shedding the organism, animal density, cow-to-calf transmission, environmental conditions/vehicles, stress, etc. (Schoenbaum & Meyer, 1995). Increased herd sizes have also made the traditional and formerly preferred practice of depopulation with indemnity more costly for the government to implement and more controversial from an environmental and animal welfare perspective (USDA/APHIS/VS, 2009d). When

only one or two animals are diagnosed with bovine TB in herds that (often) exceed 1,000 animals, VS has had a difficult time justifying depopulation (USDA/APHIS/VS, 2009d). For example, large dairy herds were discovered with bovine TB in Texas and New Mexico in the 1985 (the El Paso milk shed) and complete depopulation was not recommended because of budgetary considerations and lack of funds to compensate the herd owners for the loss of their animals. Instead the dairies were placed under individual herd plans with test and removal management plans (Whipple & Palmer, 2000*; Schoenbaum & Meyer, 1995). Despite regular testing and the removal of animal with positive results, with a larger herd, it is more likely that truly infected animals will be missed on testing; therefore, a larger herd requires more tests to eliminate all infected animals than a small herd (Schoenbaum & Meyer, 1995; Whipple & Palmer, 2000*). Large herds are also more likely to contain more anergic animals (infected cattle in a state of depressed cell-mediated immune response to tuberculin) than smaller herds (Schoenbaum & Meyer, 1995; de la Rua-Domenech *et al*., 2006*). If infected cattle are not detected (false negatives) and not removed from the herd, they can continue to transmit infection to other animals in the herd (Schoenbaum & Meyer, 1995*; Whipple & Palmer, 2000).

In addition to increased herd size, various management practices in both beef cow-calf operations and dairy operations have been associated with transmission of bovine TB (USDA/APHIS/VS, 2011g). Management practices which may increase the risk of bovine TB introduction include: the introduction of new animals with unknown bovine TB status onto the premises, exposure to wildlife or other domestic animals, exposure of feed or water to wildlife or other domestic animals, offsite heifer rearing or other practices where commingling occurs, and feeding unpasteurized milk to calves (USDA/APHIS/VS, 2011g). Lastly, cattle producers have become more specialized, necessitating the frequent transport of animals between multiple premises and for long distances. The frequent movement of some classes of cattle among multiple premises has been cited by the USDA to lead to increased risks of bovine TB transmission (USDA/APHIS/VS, 2009d).

1.3.4 Outdated regulations

The lack of flexibility in the bovine TB regulations is believed to be hindering efforts to eradicate the disease (USDA/APHIS/VS, 2009d). Many of the rules governing the national bovine TB program have been in place for decades and attempts in recent years to update the program have failed due to the burdensome federal rulemaking system (USAHA, 2009b). The USDA:APHIS:Veterinary Services (VS) regulations, including bovine TB regulations, are largely written as prescriptive design standards (or "command-and-control" standards). As such, regulated entities must follow the details in the design standards. Every time design standards and requirements need to be changed in the bovine TB regulations (specifically the Title 9 of the Code of Federal Regulations (9 CFR) parts 50 'Animals destroyed because of tuberculosis' and 77 'Tuberculosis', the Uniform Methods and Rules (UM&R) (incorporated by reference), and other related regulations (e.g., 9 CFR 71 'General provisions') additional rulemaking is necessary. VS, like other regulatory agencies, encounters a complex, lengthy process to implement changes or develop new regulations. The rule making required to change the design standards, coupled with the lengthy regulatory process, has resulted in (what some believe to be) rigid and outdated requirements that cannot adapt to a changing agricultural landscape (USDA/APHIS/VS, 2009d).

1.3.5 Antiquated approaches to bovine TB disease control

Antiquated approaches to bovine TB disease control are also believed to be impeding efforts to eradicate the disease (USDA/APHIS/VS, 2009b). In order to enhance and modernize control efforts and address the concerns of States and industry stakeholders, VS has proposed and implemented substantive changes to the bovine TB regulatory framework. The following approaches to bovine TB disease control were recently changed: whole herd depopulation and indemnity as the preferred means for disease management, multi-level State statuses and State level movement restrictions, and former animal identification standards. Historically, whole herd depopulation and indemnity, multi-level State statuses and State level movement restrictions were the foundation of the U.S. control program.

Transition from whole-herd depopulation and indemnity, as the preferred means for disease management, to test and removal

Traditionally, APHIS encouraged producers to voluntarily depopulate bovine TB-affected herds to eliminate sources of infection. Regulation 9 CFR 77.7(c) encouraged whole herd depopulation by stating an accredited-free State or zone in which a bovine TB-affected herd is detected will be reclassified to a lower status unless the herd is depopulated and an epidemiologic investigation is completed within 90 days of the detection and finds no evidence that the disease has spread (CFR, 2010d). The regulations in 9 CFR Part 50 authorized APHIS to pay indemnity to owners of animals destroyed because of bovine TB (CFR, 2010a). These payments provided a financial incentive for owners to elect depopulation instead of maintaining their herd under quarantine (USDA/APHIS, 2010d).

During the summer of 2009, in response to changing conditions in the U.S., APHIS reevaluated their approach and adopted a new policy where whole herd depopulation (the use of federal funding to depopulate entire bovine TB affected herds and indemnify herd owners) would no longer be recommended as the primary management option (USDA/APHIS/VS, 2009b; USDA/APHIS, 2010d). Whole herd depopulation with indemnity is not considered an antiquated method of addressing an affected herd, as it is still recognized by the USDA as the only method that completely eliminates bovine TB from a herd (along with the animals). Rather, it being the preferred means of disease management is antiquated in the sense that it is no longer possible to implement it in some cases due to budgetary constraints (USDA/APHIS/VS, 2009d). The new approach for managing bovine TB was made available for public comment on October 5, 2009 in a July 2009 paper titled "A new approach for managing bovine TB: VS' proposed action plan." Reasons cited for the change included the fact that the costs of depopulation increased with herd sizes at a time when future indemnity funds were expected to be limited and emergency funding to be unavailable (USDA/APHIS, 2010d), the cost of depopulation, in some cases, increased beyond what the government could afford, and the devastating impact on community economic conditions that the loss of herds from depopulation has caused (USAHA, 2009b). Under the new approach, APHIS/VS will determine the best course of action for each bovine TB-affected herd by evaluating the prevalence of disease within the herd, risk of disease transmission, effectiveness of management practices and cost effectiveness. As long as

appropriate, it will manage specific bovine TB-affected herds under a test-and-remove policy in conjunction with quarantines and restricted movement of animals to limit the spread of bovine TB from these herds. Whole-herd depopulation will be implemented when data indicate that other options will not mitigate disease spread, an imminent public or animal health risk exists or it is cost-beneficial to do so (USDA/APHIS/VS, 2009b).

Transition from multi-level State Status and State level movement restrictions to a Zoning Approach
With the new approach (that was made available in October 2009), the regulations in 9 CFR 77.7(c) ("Accredited-free States or zones") were believed to present an obstacle to the effective conduct of the bovine TB program (USDA/APHIS, 2010d). Regulation 9 CFR 77.7(c) prescribed that an accredited-free State or zone must be reclassified to a lower status if two or more affected herds are detected within the State or zone within a 48-month period (4 year). As a result of the State/zone being reclassified to a lower status (usually modified accredited advanced), cattle from it were subject to testing and other requirements for interstate movement according to 9 CFR 77.10 ("Interstate movement from modified accredited advanced States or zones") (CFR, 2010e; USDA/APHIS, 2010d). More specifically, cattle from an accredited herd in these States were accompanied by a certificate stating that the accredited herd completed the testing necessary for accredited status with negative results within 1 year prior to the date of movement, and cattle from a non-accredited herd in these States that were sexually intact animals were officially identified and accompanied by a certificate stating that they were negative to an official tuberculin test conducted within 60 days prior to the date of movement (Code of Federal Regulations, 2010e).

In order to remediate the obstacle, a federal order, issued April 15, 2010, suspended the enforcement of 9 CFR 77.7(c) and 9 CFR 77.10. In regards to 9 CFR 77.7(c), APHIS said it will no longer certify and publish the bovine TB status of individual States and it will no longer downgrade an accredited-free State or zone (or any part of that State or zone), when bovine TB is found in the State/zone and the herd is not depopulated, as long as the State or zone can prove it has the appropriate plans in place to prevent further spread of the disease. The State must meet the following criteria for controlling the disease (USDA/APHIS/VS, 2010d; USAHA, 2010):

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- maintain all affected herds under quarantine,

- implement a herd plan for each affected herd to prevent the spread of bovine TB,

- implement a program to periodically test the animals under quarantine and remove and destroy those that do not test negative, and

- conduct surveillance adequate to detect bovine TB if present in other herds or species.

In regards to 9 CFR 77.10, which pertained to movement restrictions, enforcement of the rule has been suspended in modified accredited advanced States or zones if the State was previously classified as accredited free and provided the Administrator determines that the State Animal Health Officials, in cooperation with APHIS/VS Officials, in the State or zone (USDA/APHIS/VS, 2010d):

- are maintaining all affected herds under quarantine;

- have implemented a herd plan for each affected herd to prevent the spread of bovine TB;

- have implemented a program to periodically test the animals under quarantine for bovine TB and remove and destroy those that do not test negative; and

- are conducting surveillance adequate to detect bovine TB if it is present in other herds or species.

Suspension of the rule removes Federal movement restrictions and testing obligations from cattle not affected by bovine TB in certain States where bovine TB has been found; consequently, producers in MAA States/zones whose herds are unaffected by bovine TB regain the ability to move their cattle interstate without testing for bovine TB (USDA/APHIS/VS, 2010b). Suspension allows producers to conduct business as usual if their herd is not affected by bovine TB, while only the ones with affected herds would face more stringent requirements (USDA/APHIS/VS, 2010b; USDA/APHIS/VS, 2010d). In conclusion, the Federal Order is an interim measure until regulations can be amended, it is meant to minimize the 'negative' impacts of the existing bovine TB program and update the bovine TB eradication program to match the needs of today's producers (USDA/APHIS/VS, 2010b). VS has explained that replacing the State status system with a risk-based approach, that imposes testing requirements and movement restrictions that associate with a zone, rather than an entire State, is consistent with OIE (World Organization for Animal Health) standards (USDA/APHIS/VS, 2009d).

Application of animal identification standards to meet animal identification needs

The USDA and USAHA have mentioned that the systems in place in the U.S. to trace cattle found infected with bovine TB at slaughter to a herd of origin could be improved (USAHA, 2009b; USDA/APHIS/VS 2009b and 2009d). In January 2011, the USDA published "Animal Disease Traceability Framework, Comprehensive Report and Implementation Plan" (USDA, 2011f) and in August 2011, Agriculture Secretary, Tom Vilsack, announced a proposed rule that would establish minimum national official identification and documentation requirements for the traceability of livestock moving interstate (UDSA/APHIS, 2011e). Unless specifically exempted, livestock (belonging to species covered by the rule) that are moved interstate would have to be officially identified and accompanied by an interstate certificate of veterinary inspection or other documentation, such as owner-shipper statements or brand certificates (USDA/APHIS, 2011d). Approved forms of official identification for each species, such as metal eartags for cattle, are detailed in the regulations but other forms of identification, such as brands or tattoos, would also be allowed, as agreed upon by animal health officials in the shipping and receiving States or Tribes (Hoff, 2011; USDA/APHIS, 2011d). The rules will only apply to livestock moved interstate, encourage the use of low cost technology, and will be administered by states and tribal nations (USDA/APHIS, 2011d). The purpose of the rulemaking was to improve the U.S.'s tracing capabilities, to alleviate current concerns that included the increasing number of cases of bovine TB, and to ensure preparedness to respond to new or foreign animal diseases in the future (UDSA/APHIS, 2011e).

1.3.6 Flat or decreasing budgets

Fiscal responsibility for the bovine TB eradication program is shared between federal and state governments (USDA/APHIS/VS, 1989; Code of Federal Regulations, 1992; USDA, 1992a). Nonfederal funding can include industry contributions and federal funding includes appropriated funding and emergency funding from Commodity Credit Corporation requests or APHIS contingency funds (USDA/APHIS/VS, 2009d). An exorbitant amount of money has been paid towards the program to date. At its onset, the cost of the program to livestock producers was estimated to be approximately $40 million annually by the USDA (Olmstead & Rhode, 2004) and for the 28 years between 1917 and 1945, 3,891,950 tuberculin reactors were slaughtered at a cost of $250,000,000 to the involved federal, state, and local

governments (Feldman, 1947). The cost of the program over the period 1917 to 1962 was estimated to be $258 million in 1918 dollars and $3 billion in 2003 dollars (Olmstead & Rhode, 2004). In 2006, in 2003 dollars the total cost of the control and eradication effort was estimated to have exceeded $5 billion (Gilsdorf *et al*., 2006a). The benefits of the program, in terms of public health and disease prevention, have greatly outweighed the costs, with the net annual benefits approximated at $159 million in 2006 and over $13 billion returned to the economy since the program's conception (Gilsdorf *et al*., 2006b).

The VS department expects control and eradication of bovine TB in the U.S. to be increasingly fiscally constrained at the Federal and State level (USDA/APHIS/VS, 2009d). Federal budget deficits are forecasted to continue and federal emergency funds are not anticipated to be available in the future (USDA/APHIS/VS, 2009d). State resources are expected to face similar limitations. State revenue dedicated to supporting bovine TB surveillance and eradication cannot be expected to increase and is likely to decrease (USAHA, 2009b). Federal appropriated annual funds for the bovine TB program grew to $15 million in 2003 and the USDA expects them to remain constant at $15 million or decrease (USDA/APHIS/VS, 2009d). In regards to emergency funding, the costs of investigation, control, and eradication activities have exceeded the appropriated program budget since 2001, requiring $207 million to be provided (USDA/APHIS/VS, 2009d). Due to forecasted fiscal challenges and limitations, VS has stated that future bovine TB program activities will require careful prioritization (USDA/APHIS/VS, 2009d).

1.3.7 Limitations of available diagnostic tests

1.3.7.1 Limitations of slaughter surveillance

The primary disease finding tool for bovine TB in the U.S. is slaughter surveillance (USDA/APHIS/VS, 2011g); however, there are two major limitations with the process. First, there is a general consensus that post-mortem examination has a low sensitivity for detecting bovine TB lesions (Corner, 1994; Collins, 1997; Martin *et al*., 2003; Frankena *et al*., 2007; USDA/APHIS/VS, 2009c). This means there is a low probability of detecting bovine TB at slaughter in an animal should a bovine TB lesion be present (USDA/APHIS/VS, 2009c). More specifically, the probability of detecting an animal infected with bovine TB at slaughter is dependent on the probability of an infected animal having a visible lesion, the lesion

being detected by slaughter inspectors, the lesion being submitted to the laboratory, and the laboratory testing the sample and finding it to be positive (USDA/APHIS/VS, 2009c). Estimates of the sensitivity of slaughter surveillance have ranged from 28.5% (USDA/APHIS/VS, 2009c) to 47% (Corner, 1994) and vary by country. Due to the low sensitivity, slaughter surveillance is only able to detect an unknown fraction of all bovine TB cases that exist.

The second major limitation of slaughter surveillance is that the reliance on macroscopic lesions for detecting disease means the disease is not identified until late in the course of infection, when an animal has had time to develop a lesion. Additionally, bovine TB is not identified in the herd until a successful trace-back investigation is completed, by that time the herd may be in advanced stages of disease. The probability that a surveillance system will detect infected herds depends on the following, all of which take time: the probability that the infected animal is sent to slaughter, has a lesion, the lesion is identified at slaughter and submitted to the laboratory, confirmed *M. bovis* positive, traced back to a herd of origin, and the herd is confirmed positive (USDA/APHIS/VS, 2009c). In contrast, routine periodic area wide testing, can identify infection in cattle earlier in the course of infection (prior to the development of a lesion) and in the herd prior to an advanced stages of infection (without the added time of an infected animal remaining in the herd until it has reached its time of slaughter and without the added time of conducting an epidemiological trace-back investigation).

Carcass inspection and lesion submission limitations

For 2002 to 2010, commercial slaughter in the U.S. averaged 34 million head (approximately 80% were steers and heifers and 20% were cull beef or dairy cows) (USDA/ERS, 2011). In post mortem examination, the detection of lesions suspect of bovine TB are subject to the following constraints (Corner, 1994 and 1997; Collins, 1997; Frankena *et al.*, 2007):

    1) the size of lesion,

    2) the frequency and dispersion of the lesions,

    3) environmental factors and conditions in the establishment, and

    4) human factors.

Although not specifically mentioned by Corner, Collins, and Frankena *et al.*, time has a profound effect on the detection of lesions suspect of bovine TB.

If the size of lesion is not large enough to be seen with the un-aided human eye it can be missed, no matter how careful the examination (Anderson, 1959b). Also challenging is the fact that not all infected cattle develop a gross (visible) bovine TB lesion(s). This situation is referred to as "no gross lesions (NGL)" and it often the predicament with early stages of infection. Disease experts stress that cattle who react to the tuberculin test and show NGL may be infected and should not be considered non-infected. Experience with NGLs being present has shown that tuberculin skin testing is more reliable than post-mortem results as a means of detecting infection (Anderson, 1959b). Reports have indicated that the sensitivity of the CFT test ranges from 63.2-99.9% (Monaghan *et al.*, 1994; de la Rua-Domenech *et al.*, 2006) while the sensitivity of post-mortem examination for bovine TB only ranges from 28.5-47% (Corner, 1994; USDA/APHIS/VS, 2009c).

The frequency and/or dispersion of the lesions may also affect their detection. In slaughter establishments, smaller lesions may be missed due to the difficulty of fine slicing of the lymph nodes in situ (in position) (Corner, 1994). Fine slicing of tissues may not yield a lesion if the right cut is not made to identify the lesion. Environmental factors and conditions in the establishments that can decrease the probability of identifying a gross lesion may include line speed and the light intensity (Corner, 1994).

A combination of human factors can hinder the detection of gross lesions. The quality of inspection is dependent on the knowledge, experience, motivation, diligence, autonomy, and workload of the individuals conducting the exam. In general, inspection personnel may confuse visible Mycobacteriosis compatible lesions with lesions caused by actinomycosis ("lumpy jaw"), actinobacillosis ("wooden tongue"), coccidiodomycosis ("Valley fever"), paratuberculosis ("Johne's disease"), Rhodococcus spp. (in the form of a pyogranuloma), Nocardiosis, neoplasms, parasitisms, etc. (Schoenbaum and Meyer, 1995; Gilsdorf e*t al.*, 2006; USDA/ NVSL, 2010) and smaller lesions may be missed when there is limited time available for the examination of each tissue (Corner, 1994). Specific to lesion detection and submission in the U.S., Dr.

Wagner described a number of potential reasons for the low submission rates seen in the 70's and 80's at the Tuberculosis Eradication Seminar in Ames, Iowa on July 19, 1988. The majority of reasons were related to inspection personnel. In his report, he explained that sometimes even the most skilled veterinarians cannot properly differentiate a gross tuberculosis granuloma from the multitude of other possible granulomas (Wagner, 1988). Additionally, he stated that the lack of submissions was a "people problem" where judgment, embarrassment or pride can play a part in submissions. Since bovine TB lesions vary extensively with respect to appearance and location, guidelines are used to train veterinarians to look for certain characteristics of disease. However, in order to validate that lesions are bovine TB histopathology, PCR and/or culture need to be performed. Veterinarians who are overly confident of their ability to diagnose bovine TB grossly may unintentionally ignore lesions that actually are bovine TB (Wagner, 1988). The report also mentioned the fact that the submissions of the inspecting veterinarian, of what they think are tuberculous, can set into motion events that can discourage identification (and subsequent submission) of lesions suspect of bovine TB by their subordinate meat inspectors. Additionally, the fact that carcasses must be retained until a laboratory diagnosis is obtained can induce pressure on the inspecting veterinarian from plant management if he/she retains many carcasses that do not prove to have bovine TB, particularly in plants where cooler space for carcasses is limited. Other reasons why lesions are not submitted, listed by Wagner in 1988, included (Wagner, 1988):

- Lethargy and apathy on the part of some meat inspectors,

- Lack of motivation by supervisors,

- Lack of support by hierarchy for sample collection,

- No incentive, (now resolved with monetary awards)

- Lack of recognition for his efforts when a meat inspector does submit,

- Not entirely sure of what is expected of him/her, unaware of precisely what he/she is to do with the tissues,

- Poor local liaison with Veterinary Services (VS) Meat and Poultry Inspection (MPI) Operations, and

- No VS feedback on previous submissions.

Conversely, Wagner listed reasons why personnel do submit suspect lesions (Wagner 1988):

- Greater interest in disease conditions in general,

- Greater professionalism,

- Receive positive feedback on their actions, which stimulates more submissions,

- Support from supervisors,

- Good self image, and

- Cash award.

Lastly, time effects the detection of lesions at slaughter. The more time infection has to progress in cattle, the more likely a visible lesion will develop and be found. Cattle in early stages of infection may not have macroscopically visible lesions, making detection difficult or impossible.

Lesion submission rates in the U.S.

USDA guidelines require that slaughter surveillance in the U.S. should be sufficient to detect a 0.05 percent or lower prevalence with 95 percent confidence (USDA/APHIS/VS, 2011g). In order to validate bovine TB slaughter surveillance, slaughter plants are required to submit suspicious lesions from at least 1 in every 2,000 (or 5 in every 10,000) adult cattle slaughtered; however, the frequency of granuloma submission by slaughter establishment from year to year has been highly variable in the past, ranging from 0 per 10,000 carcasses processed to greater than 30 per 10,000 carcasses processed, raising concerns about the validity of slaughter surveillance in the U.S. (Gilsdorf *et al.*, 2006a). In 1987, the Assistant Deputy Administrator of the USDA:APHIS's Veterinary Services (VS) emphasized that surveillance through slaughter sample submission must be improved and noted that of the 120 slaughter plants in the U.S. that killed over 10,000 or more adult cows annually, 40 plants had not made one submission in fiscal year (FY) 1987 (Nelson, 1987). In 1989, the 4,468 total specimen submissions represented only 34% achievement of the optimum rate (Essey & Koller, 1994).

In 2000, the Secretary of Agriculture declared an emergency in connection with the eradication of bovine TB from the U.S.; consequently, funds were provided to APHIS to allow for a more rapid and complete response to the bovine TB outbreaks, and the implementation of the Enhanced Granuloma Submission Program was proposed (USDA/APHIS/FSIS, 2000). Under the enhanced program, APHIS personnel

would be available at each of the top forty cow slaughter plants to assist FSIS inspecting veterinarian's in submitting granulomas to the National Veterinary Services Laboratory (NVSL). The APHIS personnel would not make decisions about which lesions should be collected, the FSIS veterinarian would continue to make those decisions, but would help fill out the appropriate forms (aside from signing them) and handle, package and ship the samples (USDA/APHIS/FSIS, 2000). In 2001, concerns surrounding the submission rates were expressed by Dr. Robert Meyer, National bovine TB Surveillance Coordinator for VS, in a USDA/APHIS/VS journal titled "TB Surveillance". Based on Meyer's assessment of the status of the program, he explained that efforts need to be implemented on a national basis now to drastically increase the numbers of suspicious granulomas submitted to the laboratory for bovine TB surveillance, especially from adult cattle (USDA/APHIS/VS, 2001a). The journal also stated that the Comprehensive Strategic Plan for the Eradication of bovine TB implemented by APHIS was a signal of the immediate need to enhance surveillance of cattle at slaughter in order to identify any remaining pockets of infection and ensure other areas were truly disease free (USDA/APHIS/VS, 2001a). Meyer was further quoted as stating the slaughter surveillance "method of detecting new cases of bovine TB was economical and effective as long as sufficient numbers of suspicious lesions were submitted to detect bovine TB at the very low prevalence level that exists today"; "in recent years, however, the number of suspicious granulomas being submitted for diagnosis from adult, slaughter cattle had greatly decreased" (USDA/APHIS/VS, 2001a). Noting that only 436 submissions were made from adult cattle for the year 2000, Meyer cautioned that the current level of granulomas submitted may not allow the few remaining bovine TB infected herds in the U.S. to be detected in time to reach the national goal of bovine TB eradication in domestic livestock by December 31, 2003 (USDA/APHIS/VS, 2001a).

In order to increase the quality and number of laboratory samples submitted by FSIS personnel, VS' Memorandum No. 540.6, on January 24, 2001, revised the special performance awards program for bovine TB eradication previously outlined in APHIS Directive 540.6 (June 18, 1996) and authorized the issuance of awards to FSIS inspectors and veterinarians assigned to federally inspected cattle slaughter establishments for their contributions to the eradication of bovine TB. The financial incentives for FSIS

inspectors and veterinarians, based on the results of the lesion submissions, were detailed as follows (USDA/APHIS/VS, 2001b):

> 1) a cash award of $100 for a steer or $500 for an adult animals to be shared equally each time Mycobacteriosis is reported on histopathology by the NVSL,
>
> 2) if the specimen is positive for *Mycobacterium tuberculosis* (complex) on PCR test or *M. bovis* is isolated, the cash award will be increased to a total of $200 for steers and fed heifers and $1,000 for adult animals,
>
> 3) a second cash award of $6,000 to be shared equally when an infected herd located in the U.S. is initially found as a result of the information provided to VS regarding the identification of the lesioned animal, and
>
> 4) a team award of $300 per team member awarded annually to high submitting FSIS slaughter inspection groups irrespective of histopathology results.

In 2004, FSIS revised Directive 6240.1, Bovine Mycobacteriosis Disposition Guideline (dated 11/10/94), to modify the instructions that Inspection Program Personnel (IPP) are to follow in the inspection, disposition, and sampling of cattle carcasses for bovine TB (USDA/FSIS, 2009a). IPP were directed to collect a minimum of one suspicious lesion per 2,000 adult cattle killed and to continue to collect samples from all cattle with suspicious lesions (USDA/FSIS, 2009a). The directive also mentions that FSIS will verify that the slaughter establishment collected all man-made identification devices from sampled cattle and samples are not to be sent to VS approved laboratories when no suspicious lesions are present.

Sustained efforts over the past ten years by USDA APHIS and FSIS to improve granuloma submission rates have continued to show significant and productive results with the number of granuloma submissions increasing each year. Table 2 shows the number of bovine TB suspect granulomas submitted annually for diagnostic testing for 2000 to 2010. Since FY 2000 when 436 granuloma lesions were submitted, the number of granuloma submissions has increased to 10,914 in FY 2010 (USAHA, 2010; USDA/APHIS/VS, 2011g). Many establishments substantially exceed the minimum submission rate (United States Animal Health Association [USAHA], 2008); however, as of 2010 not all plants met the minimum standard of five granulomas submitted per 10,000 adult cattle slaughtered annually (USAHA, 2010).

Table 2: Number of bovine TB suspect granulomas submitted for diagnostic testing, FY 2000-2010

Fiscal Year[a]	2000	2001	2002	2003	2004	2005	2006	2007[b]	2008	2009[b]	2010
No. of granulomas submitted for diagnostic testing	436	2,030	3,147	3,900	6,367	9,439	9,334	10,286	10,666	7,683	10,914

[a]Fiscal year = October 1 through September 31
[b]As of the third quarter of the fiscal year, Oct 1 through June 30
(Sources: USAHA 2004, 2005, 2006, 2007, 2008, 2009a and 2010; USDA/APHIS/VS, 2009e and 2010c)

National granuloma submission rate for adult cattle

The national granuloma submission rate for adult cattle (seen in Table 3) has increased from less than 1 submissions (0.76) per 10,000 adult cattle killed in 2000 to 15.1 per 10,000 in 2009, exceeding the target of 5 per 10,000 (USDA/APHIS/VS, 2009e). The number of herds in the U.S. from which these lesions came from is unknown under the current system; however, in 2009, there were 950,000 cattle (beef) operations (USDA/National Agricultural Statistics Service (NASS), 2010a) and 65,000 milk cow operations in the U.S. (USDA/NASS, 2010b). The total number of cattle operations in the U.S. has decreased 28 percent between 1989 and 2009 (USDA/NASS, 2010a) and the number of milk cow operations has decreased 33 percent between 2001 and 2009 (down from 97,460 in 2001) (USDA/NASS, 2010b).

Table 3: National granuloma submission rate for adult cattle at the end of each year, FY 2000-2009

Fiscal Year[a]	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Submissions per 10,000 slaughtered	0.76	3.29	5.1	6.85	9.29	16.2	16.4	16.6	16.3	15.1

[a]Fiscal year = October-September
Note: Target is 5 per 10,000 adult cattle slaughtered
(Source: USDA/APHIS/VS, 2009e)

Forty largest capacity (highest volume) adult slaughter establishments

Forty slaughter establishments (located in only 19-20 states) slaughter 95% of all adult cattle slaughtered in the United States (USAHA, 2004, 2005, 2006, and 2010). These plants play a critical role in all the national animal disease surveillance programs. Figure 2 shows the proportion of these 40 highest volume adult cattle slaughter establishments that met or exceed the submission standards by the end of the fiscal year, 2004-2010. The number of slaughter plants meeting the submission standard increased remarkably between 2004 and 2005 from 53% to 87.5% of the 40 highest volume adult plants. Since 2005, the number

of plants meeting or exceeding the submissions standards has remained relatively constant at about 87.5%

of 40 (or 35 plants) and 12.5% (or 5 plants) have consistently not met the submission standard.
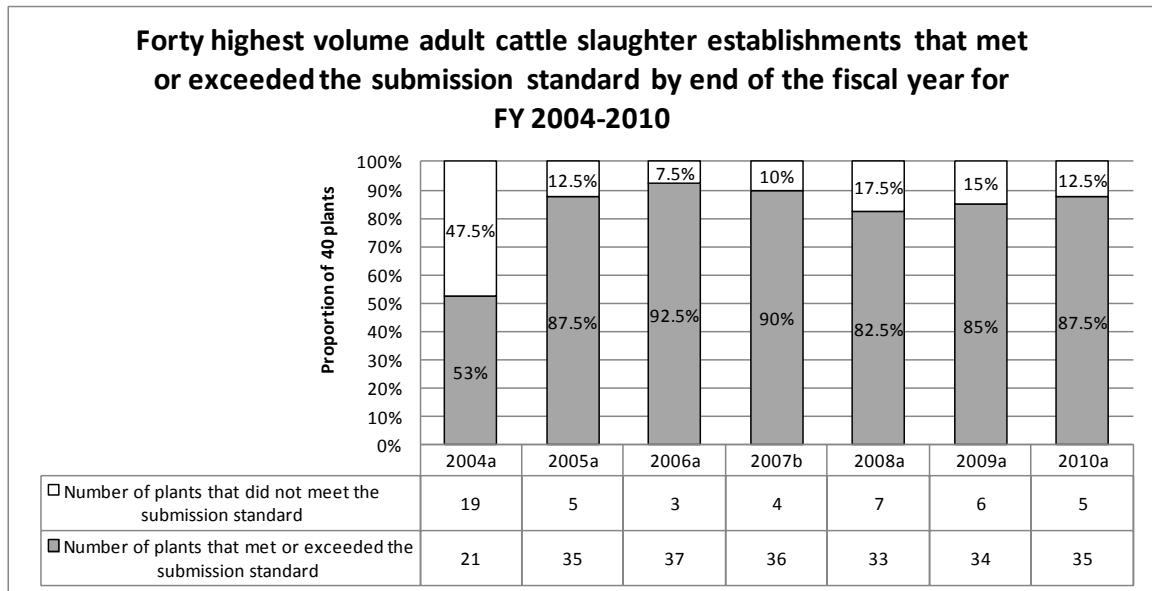
Figure 2: Forty highest volume adult cattle slaughter establishments that met or exceeded the submission standards by the end of the fiscal year, FY 2004-2010

[a] Fiscal year = October-September
[b] As of the third quarter of the fiscal year, through June 30.
Note: Target rate was 5 submissions per 10,000 adult cattle killed; compliance with the granuloma submission standard is defined as achieving greater than or equal to 85 percent of target rate (equates to 4.25 submissions per 10,000).
(Sources: USAHA, 2004, 2005, 2006, 2007, 2008, 2009a and 2010)

Table 4 shows the combined rate of submission per 10,000 for the proportion of the 40 highest volume

adult cattle slaughter plants that met or exceeded the submission standard and did not meet the submission

standard by the end of fiscal year 2004.

Table 4: Forty highest volume adult cattle slaughter establishments that met or exceeded the submission standard by the end of fiscal year 2004

Fiscal Year[a] 2004	Number of slaughter establishments	Combined rate (submissions per 10,000)
Slaughter establishment met or exceeded submission standard	21 (53%)	14.1
Slaughter establishment did not meet submission standard	5 (13%)	4
Slaughter establishment did not meet submission standard	14 (34%)	1.49
Total	**40**	**0.05-24.71**

[a]Fiscal year = October-September
Note: Target rate was 5 submissions per 10,000 adult cattle killed; compliance with the granuloma submission standard is defined as achieving greater than or equal to 85 percent of target rate (equates to 4.25 submissions per 10,000).
(Source: USAHA, 2004)

For 2004, 21 (53%) of the 40 plants had a combined granuloma submission rate of 14.1 submissions per 10,000 adult cattle killed (greatly exceeding the submission standard of 5 submissions for every 10,000 head of adult cattle killed), 5 (13%) of these plants had a rate of at 4 per 10,000 (close to the submission standard), and 14 (34%) had a rate of only 1.49 submissions per 10,000. Two of these 14 plants made only 1 submission each and killed 347,388 adult cattle. Additionally, 12 of these 14 lower-submitting plants were located in 12 Accredited-Free states, raising concerns regarding the adequacy of slaughter surveillance to effectively identify bovine TB infection in these states (USAHA, 2004). Accredited-Free states, having a low prevalence of bovine, are not likely to submit numerous suspicious bovine TB lesion; however, the plants in these states should more often than not meet the designated minimum standard (of 85% of 5 per 10,000 or 100% of 4.25 per 10,000) which is reflective of the prevalence of ACTI (actinobacillosis and actinomycosis) (Gilsdorf *et al*., 2006). Submitting below the minimal standard may be an indication that commitment is waning in that state with regards to finding bovine TB (Gilsdorf *et al*., 2006). Gilsdorf *et al*. (2006) explains that failure to meet the standard indicates that the inspection of carcasses for bovine TB may be inadequate and inadequate inspection of carcasses could delay the successful eradication of TB in the U.S. Among the 40 plants, bovine TB granuloma submission rates per 10,000 adult cattle killed ranged from 0.05 to 24.71 for FY 2004 (USAHA, 2004).

Table 5 shows the combined rate of submission per 10,000 for the proportion of the 40 highest volume adult cattle slaughter plants that met or exceeded the submission standard and did not meet the submission standard by the end of fiscal year 2005.

Table 5: Forty highest volume adult cattle slaughter establishments that met or exceeded the submission standard by the end of fiscal year 2005

Fiscal Year[a] 2005	Number of slaughter establishments	Combined rate (submissions per 10,000)
Slaughter establishment met or exceeded submission standard	35 (87.5%)	16.5
Slaughter establishment did not meet submission standard	3 (7.5%)	4.1
Slaughter establishment did not meet submission standard	2 (5%)	0.9
Total	**40**	**0.00-77.8**

[a]Fiscal year = October-September
Note: Target rate was 5 submissions per 10,000 adult cattle killed; compliance with the granuloma submission standard is defined as achieving greater than or equal to 85 percent of target rate (equates to 4.25 submissions per 10,000).
(Source: USAHA, 2005)

For 2005, 35 (87.5%) of the 40 had a combined granuloma submission rate of 16.5 submissions per 10,000 adult cattle killed (greatly exceeding the standard), 3 (7.5%) had a rate of 4.1 per 10,000 (close to the submission standard) and 2 (5%) submitted at a combined rate of only 0.9 submissions per 10,000 adult cattle killed. Among these 40 plants, bovine TB granuloma submission rates per 10,000 adult cattle killed ranged from 0.00 to 77.8 for 2005 (USAHA, 2005).

For 2006, in Figure 2 above, 37 (92.5%) plants met the criteria of compliance with the granuloma submission standard, defined as achieving greater than or equal to 85 percent of the target rate and 3 (7.5%) plants achieved less than 85 percent (83%, 74% and 52%). Among the 40 plants, bovine TB granuloma submission rates per 10,000 adult cattle killed ranged from 2.1 to 29.5 (USAHA, 2006). During 2007-09, 82.5-90% of the top 40 adult cattle slaughter plants were meeting or exceeding the target submission rate of 5 lesions per 10,000; however, 10-17.5% were submitting less than 5 lesions per 10,000 (USDA/APHIS/VS, 2007a, 2008a, and 2009a). States with plants that submitted 5 or more lesions per 10,000 killed during 2009 included California, Washington, Oregon, Idaho, Utah, Arizona, Texas, Nebraska, South Dakota, Minnesota, Wisconsin, Michigan, Ohio, Pennsylvania, Georgia, South Carolina, North Carolina and Florida. States with plants that submitted less than 5 lesions per 10,000 killed included

Wisconsin, Nebraska, Missouri, Kansas and Texas (USDA/APHIS/VS, 2010c). Of note, the slaughter plants in States where bovine TB has been found previously, like CA, MN and MI (with downgraded State statuses), the standard submission rate was met or exceeded (USDA/APHIS/VS, 2010c). For 2010, 35 (87.5%) establishments met or exceeded the submission standard and 5 (12.5%) did not (USAHA, 2010).

1.3.7.2 Limitations of tests used in the U.S.

The limitations of each bovine TB tests used in the U.S. are well-known and well-recognized. The primary downfall of the tuberculin skin test and all other available diagnostic tests for bovine TB is that they fail to detect all infected cattle, especially in populations with low-disease prevalence (USDA/APHIS/VS, 2009d). Due to the limitations with the current bovine TB tests, there is considerable need for improved diagnostic methods for bovine TB; however, significant breakthroughs in developing new tests are not likely in the immediate future (USDA/APHIS/VS, 2009d). Advances in the science have led to the development of antibody based TB diagnostic tests that have demonstrated promising results; however, the antibody tests appear more promising in cervids than in cattle (Gaborick *et al.*, 1996; USAHA, 2011). Regarding the tuberculin test, Bang (1892) indicated: "the tuberculin test is no more perfect than most things in this world. Sometimes it fails, but it would be the greatest folly to reject this method because it is not able to give everything we desire."

Single intradermal tuberculin (SIT) test limitations (in reference to both the CT and CFT test)

Tuberculin skin testing was first recognized as a useful diagnostic tool in the late 1800's and continues to be the primary diagnostic tool in both human and veterinary medicine; however, the test has limitations (USDA/APHIS/VS, 2009d). Two disadvantages of tuberculin skin testing are that the test requires two visits and the cattle must be handled twice. The veterinarian must visit once to administer the injection and again 72 hours later to interpret the results (USDA/APHIS, 2005a; USDA/APHIS/VS, 2009d; USAHA, 2009b). The efficacy of tuberculin testing is dependent on (Gilsdorf *et al.*, 2006a):

- the competency and honesty of state, federal, and accredited veterinarians to correctly classify reactions, and
- the inherent ability of the test to detect infection.

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In order to classifying reactions correctly, the veterinarian injecting and reading the test must be adequately trained to inject the tuberculin and interpret the test. Interpreting a "response" may be subjective, particularly if the response is small (USAHA, 2009b). In regards to human factors, false negative results for tuberculin skin testing can be due to administrator error or the underreporting of reactions (Monaghan *et al*., 1994).

Multiple field trials have been conducted to estimate the inherent ability of the SIT (CT and CFT) test to detect infection in cattle. Table 6 is a summary of the results of trials conducted to estimate the sensitivity and specificity of the SIT test in cattle in chronological order or publication. In 1973, results of a literature review conducted by USDA researchers concluded that the sensitivity of the SIT (proportion of infected cattle that test positively) procedure was 85% and the specificity (proportion of non-infected cattle that test negatively) was found to be 95-98% in various U.S. cattle studies (Roswurm & Konyha, 1973; Meyer, 1988). Published summaries of field trials have reported that the sensitivity of the single tuberculin test ranged from 68-87.9% (Monaghan *et al*., 1994) and from 63.2-100% (with a median of 83.3%) (de la Rua-Domenech *et al*., 2006). The specificity has been reported to range from 90.8-98.8% by Monaghan *et al*. (1994) and 75.5-99.0% (with a median of 96.8%) by de la Rua-Domenech *et al*. (2006). As a reasonable guide for the SIT test is that the sensitivity is 85% and the specificity is 97%; consequently, as many as 15% of the infected cattle will falsely test negative to the single intradermal tuberculin skin test (due to the sensitivity) and approximately 3% of uninfected cattle may falsely test positive (due to the specificity) (USDA/APHIS/VS, 2009d).

Table 6: Estimated sensitivity and specificity of the single intradermal tuberculin (SIT) test in cattle

Type of tuberculin test	Reference (country)	Sensitivity (%)	Specificity (%)
Caudal fold SIT	Francis *et al*., 1978 (Australia)*	72.0	98.8
	Wood *et al*., 1991 (Australia)*	63.2	99.0
	Wood *et al*., 1992 (Australia)*	68.2	96.8
	Whipple *et al*., 1995 (USA)*	80.4-84.4	Not evaluated
	Norby *et al*., 2004 (USA)*	83.3 & 96.8	Not evaluated
	Median (%)	**81.9**	**98.8**
Cervical SIT	Lesslie *et al*., 1975 (GB)*	100	88.6
	Averages of six sources[a] from various countries, (cited in Francis *et al*. 1978))*	91.2	75.5
	Domingo *et al*., 1995 (Spain)*	91.0	Not evaluated
	Gonzalez Llamazares *et al*., 1999 (Spain)*	80.2	Not evaluated
	Pollock *et al*., 2003 (Northern Ireland)*	86.4	90.0
	Cagiola *et al*., 2004 (Italy)*	Not evaluated	96.8
	Median (%)	**91.0**	**89.3**
SIT	Roswurm & Konyha, 1973	85.0	95.0-98.0
Overall median (%) for SIT		**84.4**	**96.8**

[a] Kerr *et al*. (1946); Kerr *et al*. (1949), Van Waveren (1953), Paterson *et al*. (1958), De Jong and Ekdahl (1969) and Lessile *et al*. (1975), all cited in Francis *et al*. (1978).
*Adapted from de la Rua-Domenech *et al*., 2006. For quantity of bovine PPD, number of animals tested (of which necropsied), and apparent proportion diseased (%), see de la Rua-Domenech *et al*., 2006.

A "false-negative" result, due to the test's incomplete sensitivity, enables a bovine TB infected animal to remain in the herd, with the potential to spread the infection (Goodchild & Clifton-Hadley, 2001). Factors which may result in false negative reactions have been reported by various authors. It has been reported that the sensitivity of the SIT test (and proportion of false negatives) is affected by the injection site, preparation, potency and dose of tuberculin units (TU) administered, its inability to distinguish between latent stages of infection and disease (those tested too early or too late in the course of infection), desensitization from previous tuberculin test(s), deliberate interference, immunosuppression and characteristics of the immune system of the population of animals being tested, and the cut-off value used to define a positive result (Costello *et al*., 1997; Monaghan *et al*., 1994; Schoenbaum & Meyer, 1995; de la Rua-Domenech *et al*., 2006; USDA/APHIS/VS, 2011g). Reportedly, the most sensitive tuberculin test is the CT test (10,000 TU), followed by the CFT test (5000 TU) and the least sensitive, the CCT test (Schoenbaum & Meyer, 1995). After being infected with bovine TB, cattle traditionally experience a latency period during which they are unresponsive to testing; the length of this unresponsive period can vary between 8 and 65 days for the SIT test (Barlow *et al*., 1997; Kao *et al*., 1997; Kleeberg, 1960). Some cattle that have been infected for a long period may not show any reaction to tuberculin skin testing, due to

being in a state of depressed cell-mediated immune response to tuberculin; a scenario called anergy or anergic cattle (Lepper *et al*., 1977). An example of deliberate interference is a practice known as "plugging the test", where chemicals are used in the cattle's feed, can also make cattle unable to respond to the test (Meyer, 1988). Immunosuppression may occur in certain physiological states of stress, during infection with certain diseases, during early postpartum, and in progressive bovine TB infection and may cause a diminished or undectable test reaction (Lepper *et al*., 1977; Costello *et al*., 1997). Altering the cut off value used to define a positive result, to increase or decrease the test's sensitivity, will inversely affect the test specificity (Costello *et al*., 1997). Failure to detect all bovine TB infected animals due limitations in the sensitivity of the tuberculin test can contribute greatly to the persistence of the disease contributing to the failure of some countries to eradicate bovine TB (Monaghan *et al*., 1994).

A "false-positive" result due to the lack of perfect specificity of the SIT test can result in healthy cattle being unnecessarily removed from the herd. A false positive reaction can be caused by the tests inability to distinguish between individual *M. tuberculosis* complex species, cattle exposed to *M. avium* subspecies *paratuberculosis (MAP)*, environmental Mycobacteria and skin tuberculosis can trigger a response to the SIT (Anderson, 1959b; Monaghan *et al*., 1994; de la Rua-Domenech *et al*., 2006). The SIT test has a relatively low false positive percentage with a specificity of 97%; however, the lack of complete specificity can be problematic in countries with very low levels of bovine TB (Monaghan *et al*., 1994), such as the U.S.

Single intradermal comparative cervical tuberculin (SICCT) test limitations (the CCT test)
Monaghan *et al*. (1994) and de la Rua-Domenech *et al*. (2006) summarized the results of multiple trials conducted from 1946 and 1986 and from 1973 and 2004, respectively, in various countries, and found that the sensitivity of the SICCT test was reported to range from 77-95% and from 52-100% (with a median of 80%) respectively. The specificity of the SICCT was reported to range from 94-100% by de la Rua-Domenech *et al*. (2006). Table 7 shows a summary of the trials that de la Rua-Domenech *et al*., (2006) assessed. A reasonable guide for the SICCT is that the sensitivity is 80% (for the standard test interpretation) and the specificity is 99.9%.

Table 7: Estimated sensitivity and specificity of the single intradermal comparative cervical tuberculin (SICCT) test in cattle

Reference (country)	Test interpretation	Sensitivity (%)	Specificity (%)
Roswurm and Konya, 1973 (cited by	Standard	74.4	
Norby et al., 2004)	Severe	88.5	
Lesslie et al., 1975 (SE of England)	Details not given	Not evaluated	100
Lesslie et al., 1975 (GB)	Standard	91.4	99.9
	Severe	100	
O'Reilly and MacClancy, 1975	Standard	95.5	97.8
As cited by Francis et al., 1978[a]	Severe	68.6	88.8
O'Reilly, 1986	Standard	75.0	
	Severe	94.1	
Neill et al, 1994b (Northern Ireland)	Standard	55.1	100
Doherty et al., 1995b (Ireland)	Standard	93.3	Not evaluated
	Severe	100	
Costello et al., 1997 (Ireland)	Standard	90.9	Not evaluated
Ameni et al., 2000 (Ethiopia)	Standard	90.9	100
Buddle et al., 2001 (New Zealand)	Standard	Not evaluated	94
Quirin et al., 2001 (Madagascar)	Subjective assessment	52.0	99.0
		80.0	100
Norby et al., 2004 (USA)	Severe	75.0	Not evaluated
	Severe	93.5	Not evaluated
Median (%)		**80.0 for Standard, 93.5 for Severe**	**99.9**

[a] Kerr et al. (1946); Kerr et al. (1949), Van Waveren (1953), Paterson et al. (1958), De Jong and Ekdahl (1969) and Lessile et al. (1975), all cited in Francis et al. (1978).
(Adapted from de la Rua-Domenech et al., 2006. For quantity of bovine PPD and avian PPD, number of animals tested (of which necropsied), and apparent proportion diseased (%), see de la Rua-Domenech et al., 2006.)

The SICCT test is subject to similar reasons for false positives and false negatives as mentioned above for the SIT testing. Due the injection of avian PPD tuberculin concurrent with the bovine PPD tuberculin, the specificity of the SICCT test is typically higher than the SIT test (resulting in less false positives). When testing a herd of unknown bovine TB status, testing in series with the CCT (after an animal has tested positive to the CFT) reduces costs by increasing specificity (fewer false positive animals are sent to slaughter); however, the sensitivity is decreased (and infected animals may be falsely identified as negative) (USDA/APHIS/VS, 2011g).

Gamma interferon limitations

The disadvantages of the IFN-γ test, in comparison to the tuberculin skin tests, include (Gormley, 2004; Pollock *et al*., 2005; de la Rua-Domenech *et al*., 2006; Michel *et al*., 2010; USDA/APHIS/VS, 2010c):

- the expenses and logistics related to the necessity of the blood samples to be shipped to a laboratory in less than 24 hours,

- the (sophisticated) laboratory equipment required to process the blood samples,

- the sensitivity of the test decreases considerably following delays in sample processing,

- a small proportion of bovine TB infected cattle that are reactors to the skin test may not be detected by the IFN-γ test, and

- non-infected young cattle are likely to give a non-specific response to the test.

The IFN-γ test, shares with the tuberculin skin tests, the disadvantage of having a low probability of detecting anergic cattle, leading to false negative results (de la Rua-Domenech *et al*., 2006). de la Rua-Domenech *et al*. (2006) summarized the results of multiple studies conducted from 1991 through 2004 in various countries and found that the sensitivity of the IFN-γ (Bovigam^TM) test ranged from 73.0-100% (with a median of 87.7%) and the specificity ranged from 85.0-99.6% (with a median of 96.6%). Table 8 is a summary of the published studies that de la Rua-Domenech *et al*. (2006) assessed. A reasonable guide for the IFN-γ test is that it has a sensitivity of 88% and a specificity of 97%.

Table 8: Estimated sensitivity and specificity of the gamma interferon (Bovigam^{TM}) test in cattle

Reference (country)	Criterion for test interpretation	Sensitivity (%)	Specificity (%)
Wood *et al*., 1991 (Australia)	C4	81.6	99.4
Wood *et al*., 1991 (Australia)	C1	Not evaluated	96.2
	C2		97.4
	C3		98.0
	C4		98.1
	C5		97.8
Wood *et al*., 1992 (Australia & New Zealand)	C4	81.8	99.1
Neill *et al*., 1994a (Northern Ireland)	C6	Not evaluated	99.6
Neill *et al*., 1994b (Northern Ireland)	Not stated	84.3	Not evaluated
Whipple *et al*., 1995 (USA)	C4	73.0	Not evaluated
Domingo *et al*., 1995 (Spain)	C4	93.7	Not evaluated
	C11	87.4	
Monaghan *et al*., 1997 (Republic of Ireland)	C1	87.7	Not evaluated
	C7	Not evaluated	96.6
Lilenbaum *et al*., 1999 (Brazil)	C4	100	94.0
Gonzalez Llamazares *et al*., 1999 (Spain)	C4	84.9	Not evaluated
Lauzi *et al*., 2000 (Italy)	C12	Not evaluated	88.9
Ameni *et al*., 2000 (Ethiopia)	Not stated	95.5	87.7
Ryan *et al*., 2000 (New Zealand)	C6, C13	85.0	Not evaluated
	C13	Not evaluated	93.0
Buddle *et al*., 2001 (New Zealand)	C6	94	Not evaluated
	C14	98	Not evaluated
	C6	Not evaluated	94
	C14	Not evaluated	85
Vordermeier *et al*., 2001b (GB)	C13	88.2	Not evaluated
	C13	Not evaluated	92.0
Cagiola *et al*., 2004 (Italy)	C13	Not evaluated	97.3
	C13		98.6
Median (%)		**87.6**	**97.0**

(Adapted from de la Rua-Domenech *et al*., 2006. For quantity of bovine and avian tuberculin, criterion for test interpretation, number of animals tested (of which necropsied), and apparent proportion diseased (%), see de la Rua-Domenech *et al*., 2006.)

Generally, the sensitivity of the IFN-γ test is considered to be similar to that of the SIT and greater than the SICCT test and the specificity is considered to be lower than the intradermal tuberculin tests (de la Rua-Domenech *et al*., 2006). For diagnosing bovine TB, the IFN-γ test is sometimes considered less sensitive than the CFT (Whipple *et al*., 1995; Gonzalaz Llamazares et al., 1999). The IFN-γ test may be used to classify CFT responders, or in parallel with the CFT. Combining the IFN-γ and CFT (or CCT) in parallel may improve sensitivity (Wood *et al*., 1991; Whipple *et al*., 1995; Gormley *et al*., 2003). Improving sensitivity has an inverse effect on specificity, increasing the number of false positives; therefore, parallel testing with the γ-IFN is applied in a situation where a relatively high level of false positives is acceptable in order reduce the time taken to clear the herd of bovine TB (Olea Popelka, 2007). Parallel testing is not

standard practice in the U.S. but may be conducted as part of an affected herd plan to implement a test-and-removal management approach of animals (USDA/APHIS/VS, 2011g).

Culture limitations

Culture, unlike histopathology and PCR, provides the specific genus and species when diagnosing bovine TB; however, it takes four to eight weeks. Reasons for false negatives when attempting to grow *M. bovis* from a sample include: the bacteria can be dead and hence not grow, improperly preserved tissue sample can become contaminated decreasing the number of viable *Mycobacteria* and the chemical used for transportation, sodium tetraborate, can decrease the viability of the bacteria after 72 hours of storage (Thomson, 2006). Contamination of the sample can result in false positives (Thomson, 2006).

PCR limitations

Although polymerase chain reaction (PCR) testing (for IS6110) of formalin-fixed tissues is a rapid method of diagnosing tuberculosis, it does not definitely identify the type of *Mycobacteria* present. Instead, it indicates whether the deoxyribonucleic acid (DNA) of the bacteria present (if bacteria is in fact present) in the sample are of the *Mycobacteria tuberculosis* complex (MTBC). Since several of the seven species of the MTBC are not commonly found in the U.S. and in cattle, if PCR indicates that the bacteria is MTBC then it is highly likely to be *M. bovis* (Thomson, 2006). The sensitivity of PCR can be affected (decreased, leading to false negatives) by several different scenarios. When a tissue sample, compatible for *Mycobacteriosis* by histopathology, is subdivided for PCR and culture it is possible that only one of the subsamples will contain bacteria. If the PCR sample does not contain any bacteria, a false negative will result (Thomson, 2006). Additional causes for a false negative include prolonged formalin fixation leading to DNA degradation, decalcifying solutions, and the type of tissue fixation solution (Thomson, 2006). Contamination of the sample is the leading cause of false positives (reduced specificity) for PCR (Thomson, 2006).

1.3.8 Inability to trace some infected cattle identified at slaughter back to a herd of origin

1.3.8.1 Limitations of epidemiological trace-back investigations

The epidemiological trace-back investigation conducted for cattle found at slaughter with lesions suggestive of bovine TB is the major method by which the USDA/APHIS locates newly infected herds. Not only is the success of the epidemiological trace-back investigation important but so is the timeliness for the detection of infected herds and control of the disease. Successfully tracing back to a herd of origin is highly dependent on "the collection and availability of current and accurate information about individual animals" (Kaneene *et al*., 2006). The ability to determine an animal's whereabouts from birth to slaughter is defined by the USDA/APHIS as traceability (USDA/APHIS/VS, 2008e).

Recently, concerns have been reported about the success rate of tracing animals from slaughter back to a herd of origin (Kaneene *et al*., 2006; USDA/APHIS, 2007c; USDA/APHIS/VS 2009b and 2009d; USDA/APHIS, 2011e). Kaneene *et al*. (2006) cited that the success rate of tracing bovine TB infected cattle back to a herd of origin was between 50 and 70% of the investigations undertaken. The authors expressed that the current rate was concerning, however; their suggestion was that the rate be improved only to 75%. The USDA/APHIS has recognized that they have deficiencies with regards to tracing cattle and expressed in 2007 the desire to be able to trace 70% of breeding cattle back to their premise(s) of origin and that they envisioned a 48-hour turn-around time in the future (USDA/APHIS, 2007b and 2007c). It is possible; however, that in order to control bovine TB, an investigation success rate anything short of 100% may not be good enough. To date, no studies have been conducted in the U.S. to determine an appropriate trace-back "rate" in order to control the disease. Based on the chronic nature of the disease and the ramifications of not finding the sources of bovine TB cases detected at slaughter, implementing measures to trace 100% of cattle (disclosing bovine TB lesions at slaughter) back to all of their prior premise(s) are in the country's best interest. Cost, of course is an issue, however; the cost of not being able trace cattle and find the sources of disease have the potential to outweigh the cost of the infrastructure needed to trace them (since multiple producers have to be tested when a herd of origin is not identifiable).

Some of the difficulties reported when conducting epidemiological trace-back investigations (to find the herd of origin and exposed animals) in the U.S. have been related to the following:

1) slaughter establishment procedures for carcass identification, animal identification retention, and record keeping systems (FSIS &APHIS, 1992; USDA/APHIS/VS, 2001b; USDA/FSIS, 2002),

2) lack of animal identification at slaughter, the variety of available animal identification and decreased participation in disease programs that encourage animal identification (USDA/APHIS, 2007b; USDA/APHIS/VS, 2009d; USDA/APHIS, 2011e),

3) incomplete documentation owner, dealer and broker documentation (USAHA, 2004; USDA/APHIS/VS, 2007c and 2009d),

4) extensive movement of animals (USDA/APHIS, 2008b; USDA/APHIS/VS, 2009d) with the potential for multiple herd of origin for the same slaughtered cattle (with lack of determination the source of infection),

5) length of time to conduct investigations (Wagner, 1988; USDA/APHIS/VS, 2007c and 2009d),

6) the practice of raising dairy heifers in large dry lot facilities with feeder cattle (USAHA, 2009b), and

7) identification of herds with advance stages of disease (Raney, 1967 and 1970; Meyer, 1988).

Slaughter plant carcass identification systems and record keeping

During the animal identification (ID) collection process, the animal identification has the potential to be physically separated from the carcass before the completion of viscera inspection. In 1992, Veterinary Services' history of trace-back investigations showed that "frequently" the identification present at slaughter was incorrectly correlated with infected carcasses preventing detection of an infected herd(s)(FSIS and APHIS, 1992). The "countback system", once the most commonly used system, was blamed since it was prone to errors in correlating the correct identification with the affected carcass (FSIS and APHIS, 1992). VS found from experience that there was a direct association between successful trace-backs and the accuracy of systems correlating identification devices with the correct carcass. The "countback" systems of recovering ID was found to result in about 50 percent unsuccessful trace-backs to

the correct herd of origin; subsequently, more positive carcass/ID correlation systems were highly

recommended (USDA/APHIS/VS, 2001b). Despite the errors in correlation, the infected herds may have

eventually been found but only after testing all consignees to the slaughter lot. Based on this finding, a

FSIS and APHIS bovine TB task force report recommended all FSIS federally inspected slaughter

establishments be evaluated for efficacy of ID collection and correlation with the affected carcass (FSIS

and APHIS, 1992). If an establishment was found to be deficient, recommendations were made to

remediate the establishment's system. Importantly, some establishments had very good methods for animal

identification correlation that provided a high degree of security and assurance of accuracy (FSIS and

APHIS, 1992).

A brief report completed by the FSIS in 2002 described some of the problems encountered when collecting

animal identification at slaughter at 42 meat inspectors at slaughter facilities across the U.S. (USDA/FSIS,

2002). Inspectors reported that a wide variety of identification systems were in use, tag and identification

requirements differed significantly between states, and plant recordkeeping systems were dependent on the

quality of information being received from a variety of sources (e.g., sale barns, feedlots, shippers,

producers, auction houses). Larger slaughter facilities had more formalized recordkeeping systems, while

very small plants relied on log books or paper invoices for records. The inspector reported that a

significant percentage of animals had no identification, and some plants had difficulty keeping animal ID

tags with all parts of the carcass. When animals were handled in lots rather than as individuals, there were

difficulties in counting the numbers of animals, and animals were often re-grouped so that it was

impossible to identify the source of a given animal from a specific lot (Kaneene *et al.*, 2006). The report

made several recommendations, including requiring individual animal ID for all animals entering slaughter,

development of a uniform system of identification, clarifying the roles of APHIS, FSIS, and the slaughter

plant in trace-backs, the use of electronic tracking, and sharing of databases (USDA/FSIS, 2002).

Concerning the collection of animal identification at slaughter plants, the USDA stated in January 2011,

that the new approach/regulation for animal disease traceability issued in April 2011, removed the criteria

for collecting 75 percent of identification at federally inspected slaughter plants and that "the collection of

ID at slaughter hinges more on availability of funds and is not a good indicator of whether the identification processes are working through the preharvest production chain" (USDA, 2011f). It is unclear whether is it is required in other regulations that a certain percentage of ID be collected at slaughter. Collecting less than 100% of the ID present on cattle at slaughter would detrimental to the bovine TB control and their ability to successfully tracing bovine TB lesioned animals back to their herd of origin to find sources of disease.

Animal identification issues

Since producers in the U.S. use multiple identification numbers on a given bovine for a variety of purposes (animal health programs (i.e. brucellosis tag numbers), industry-sponsored programs (herd improvement programs), or interstate commerce (i.e. bright/brite tag numbers)), matching identification numbers between different systems makes tracking cattle from slaughter back to farm slow and difficult (Kaneene *et al*., 2006). The various forms of animal identification devices used to trace animals today include the following:

1) National Uniform Eartagging System (referred to as bright or brite tags or tuberculosis tags):

Historically, these tags are used for disease testing and interstate movement. This form of identification is used when official identification is required and is not specific to any particular disease program (USDA, 2011f). The tag may be applied at any given point in an animal's lifetime (they are not necessarily applied at birth) and are primarily applied to animals destined for interstate movement. Bright tags for cattle are silver metal eartags that contain a 9 character format National Uniform Eartagging System (NUES) number (2 number state code, 3 alpha characters, followed by 4 numbers)(USDA, 2011f). Bright tags are considered official ID (USDA, 2011f).

2) Brucellosis Vaccination Eartag (Bang's tags or Official Calf-hood Vaccinate (OCV) tags):

Bang's tags are required only for certain young female cattle (aged 4 to 12 months moving into and out of states designated as Class B or C for brucellosis (USDA/APHIS, 2011h; Cattle Today, 2011). These vaccinated calves must be permanently identified by means of a tattoo, and either an official vaccination eartag or other official eartag if an official tag is already attached to the animal (9 CFR part 78) (CFR, 2010f; USDA/APHIS, 2011e). Since all 50 states are currently considered brucellosis-free (despite

the detection the disease in 5 domestic cattle and bison herds in Wyoming, Montana, and Texas in 2011(USDA/APHIS, 2011h)), the brucellosis vaccination tag is essentially voluntary (note: once vaccinated the ear tag and tattoo are required) and the number of cattle identified in this manner has steeply declined (USDA/APHIS, 2011e and h). Bang's tags are orange metal ear tags that contain a National Uniform Eartagging System (NUES) number (Bang's tags are unique from other NUES numbers in that they have a 2 number state code, V or T followed for 2 alpha characters, followed by 4 numbers) (USDA, 2011f). Bang's tags are considered official ID (USDA, 2011f).

3) Animal Identification Number (AIN) "840" Tags:

AIN tags provide a nationally unique ID number for each animal. The AIN consist of 15 digits, with the first 3 being the country code (840 for the U.S.). The 840 prefix should only be used on animals born in the U.S. (USDA, 2011f). Not all of these tags currently use the 840 prefix, some use USA or another numeric coded. The tag is provided to producers or animal health official and is available in various sizes, shapes and colors. It can be visual only or contain radio frequency identification technology. AIN tags are considered official ID (USDA, 2011f).

4) USDA approved backtag (or Market Cattle Identification (MCI)):

A back tag is a tag issued by APHIS that provides a temporary unique identification for each animal (USDA, 2011f). USDA backtags are typically applied at livestock markets. Other publications report that the backtag was intended to provide a means of determining brucellosis status of animals and is placed solely on the shoulders of breeding beef and dairy cattle that go through a livestock market (Golan *et al.*, 2004). USDA backtags are considered official ID (USDA, 2011f).

5) Mexican and Canadian animal identification tags:

Since at least 1999, blue ear tags have been applied to all Mexican origin cattle sent to slaughter in the U.S. The blue ear tags are part of the Mexican national bovine TB eradication program, contain prefixes indicating the "Mexican State of Origin" and have six unique animal identification numbers associated with each tag (USDA-APHIS/FSIS internal document by Reed, 1999). Canadian identification is animal specific and includes a prefix of CAN or EID followed by a string of unique numbers.

6) Management bangle tags:

Management bangle tags are generic non-official, non-permanent tags applied by the producers to manage a herd.

7) Registered brands and breed registry tattoos:

Brands and breed registry tattoos applied by producers can be farm and/or animal specific. Brands and registry tattoos are considered official animal identification (USDA/APHIS/VS, 2008c); however, the usefulness of them for bovine TB investigation purposes is limited because cattle hides are removed and separated from carcasses early in the slaughter process.

Official identification devices are intended to provide permanent identification of livestock and to ensure the ability to find the source of animal disease outbreaks. Removal of these devices, including those applied to imported animals in their countries of origin and recognized by the Administrator as official, is prohibited except at the time of slaughter according to CFR, Title 9, Chapter 1, Section 71, Part 22 (CFR, 2010b). However, compliance with this rule has been poor. In FY 2003, 23% of 39 cases of bovine TB had no animal identification of any type, making it difficult if not impossible to locate the herd of origin (Kaneene *et al.*, 2006). From October 1, 2003, through March 17, 2007, 156 positive cases of bovine TB were identified in the U.S. Of those cases, "11 percent of the animals had no identification whatsoever," and 83 percent of the positive cases did not have official USDA individual identification present (USDA/APHIS, 2007b). When animal identification is not present, animal health official are left to relying the descriptive characteristics of the infected animal, i.e. color, breed, live ('hot') weight, carcass weight, sex, age, and the receipts and records of the owners, dealers and brokers. When animal identification is "lost", the infected herd may never be found (Wagner, 1988).

In the past, traceability has been tied to disease control programs (USAHA, 2009b). In 2007, the USDA explained that, "The current U.S. traceability infrastructure falls short of the long-term 48-hour objective envisioned" and part of the reason for this shortfall, "As diseases have been eliminated, participation in active disease programs has lapsed-causing the traceability infrastructure [in the U.S.] to be less effective than it once was" (USDA/APHIS, 2007b). The great progress and successes of the U.S. brucellosis and bovine TB programs resulted in a steep decline in the number of officially identified cattle (USAHA,

2009b; USDA/APHIS, 2011e). In 1988, 10 million calves were officially identified for brucellosis when 27 States were "Class-free", but by 2010 with all States brucellosis-free the number identified had fallen to 3.1 million (USDA/APHIS, 2011e). As a result of decreasing levels of official identification in cattle, the time required to conduct disease investigations is increasing; disease investigations for bovine TB frequently now exceed 150 days as USDA and State investigative teams spend substantially more time and money when conducting trace-back investigations (USDA/APHIS, 2011e). The decreased level of official identification has also expanded the scope of investigations needed to identify suspect and exposed animals, at times necessitating the testing of thousands of cattle that would otherwise not have needed to be tested (USDA/APHIS, 2011e).

A new approach and rule for animal disease traceability was proposed in February 2010 and issued in April 2011. It will require cattle moved interstate to be officially identified and accompanied by an interstate certificate of veterinary inspection (ICVI) or other official documentation, with some exceptions. The new approach will be codified (made into rule) by adding a new animal disease traceability section to Title 9 of the Code of Federal Regulations. In August 2011, the USDA/APHIS disclosed their concerns with tracing cattle and proclaimed that they must improve their tracing capabilities now in order to alleviate current concerns, including the increasing number of animals with bovine TB (USDA/APHIS, 2011e). They cited that the biggest inadequacies related to identification and movement (documentation) requirements for animals for disease programs were in the cattle industry (UDSA/APHIS, 2011e). The inadequacies they refer to relate to the animal disease programs, e.g. bovine TB and brucellosis, that do not apply identification requirements to all cattle or to all cattle moved interstate (USDA/APHIS, 2011e). Prior to the new rule, only adult cattle over 24 months of age were required to be officially identified for interstate movement (9 CFR 71.18)(USDA/APHIS, 2011f and 2011h).

The new rule (issued in April 2011) addressing official ID will apply to some but not all cattle; it will only apply to cattle moving interstate. Additionally, application of the official ID for cattle moving interstate only needs to be applied before interstate movement; it will not be applied at birth. The USDA's rationale for focusing solely on interstate movement is based on the belief that interstate movement is where the

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impact of disease spread is the greatest (USDA/APHIS, 2011f). Two important concerns that are not being considered as a part of this effort are the importance of being able to trace cattle within a state and the need to apply the official ID at birth to facilitate a complete (full) trace-back investigation (to all of the premises where the animal may have resided in its lifetime).

Additionally, the new rule will not apply to all cattle moved interstate. First, the new regulation will initially (in phase/step I) include all sexually intact cattle 18 months of age or over, dairy cattle of any age, cattle of any age used for rodeo or recreational events and shows or exhibitions; however, it will initially exclude beef cattle under 18 months of age (feeder cattle) and cattle moved directly to slaughter (including through one approved livestock facility, i.e., auction/market) with a USDA approved backtag (USDA/APHIS, 2011f). There will be a three step plan to phase in the official ID requirement for beef cattle under 18 months of age (fed cattle). Step I of plan is application of official ID on dairy and adult beef breeding cattle moving interstate, phase II is an assessment to see how effective implementation of phase I has been and step III is full implementation of official ID on all ages/classes of cattle being moved interstate. Step III is suppose to take place one year after conformity with Step II, projected to be in 2015. The assessment in phase II will determine whether there is 70-percent compliance with official ID requirements for cattle in Step I; if this rate is attained the official ID requirements will then in phase III be expanded to the young beef cattle. The time it might take to attain the 70-percent compliance could be significant and greatly hinder the U.S.'s goals for animal traceability.

Second, during phase (I), cattle can be exempt from the official ID requirement when 1) they have a USDA-approved backtag, and 2) the USDA-approved backtag is applied to the cattle at the slaughter plant or federally approved livestock facility approved to handle "for slaughter only" animals, under the condition that the cattle are moved directly to a recognized slaughter establishment or directly to no more than one approved livestock facility approved to handle " for slaughter only" animals and then directly to a recognized slaughter establishment (USDA, 2011f). Application of official ID at the slaughter plant (terminal destination) or at the livestock market will not facilitate a complete (full) trace-back investigation (to all of the premises where the animal may have resided in its lifetime).

Third, during phase 1 and II and upon completion of phase III, the USDA will allow an exception to the authorized forms of official ID if cattle that are be moved between any two states or tribes have another form of identification (i.e. branding, tattoos and breed registry certificates) as agreed on by animal health officials in the two jurisdictions (USDA, 2011). Since brands are removed early in the slaughter process and are separated from the carcass, they cannot be used to facilitate bovine TB trace-back investigations.

Fourth, upon full implementation of the rule (phase 3), the new official ID interstate movement requirements would not apply to (USDA/AHPIS, 2011f):

- the movement of cattle to a custom slaughter facility in accordance with State and USDA's FSIS regulations for preparation of meat for personal consumption, and
- cattle that are moved interstate directly to an approved tagging site. (These cattle, however, must be officially identified before commingling with cattle from other premises).

Based on the above, after the full implementation of the new rule, the U.S. will continue to lack a comprehensive animal traceability program.

Incomplete owner, dealer and broker documentation

The success and completeness of trace-back investigations are dependent on the collection, availability and quality of the receipts and records about individual animals from owners, dealers and brokers (Kaneene *et al*., 2006). According to the CFR, Title 9, Chapter 1, Section 71, Part 20, livestock markets (in order to qualify as an approved facility) must maintain documents, such as weight tickets, sales slips, and records of origin, as well as, any available identification and destination information (that relates to the animals are in or have been in the facility) for a period of two years (CFR, 2010o). The APHIS representatives and State representatives are permitted to review and copy those documents during normal business hours. Incomplete owner, dealer and broker documentation has been found to limit trace-back efforts (USDA/APHIS, 2007c). For example, insufficient record-keeping by owners made trace-back to herd of origin impossible for a bovine TB-positive adult beef cow identified in FY 2004, despite the fact that the cow did have an official ear tag for identification at slaughter (USAHA, 2004).

Extensive movement of animals

The frequent movement of some classes of cattle among multiple premises and herds has complicated trace-back investigations (USDA/APHIS/VS, 2009d). Appendix B from Golan *et al*. (2004) shows the different pathways to slaughter for different classes of cattle in the U.S. It is important to note that there are numerous permutations and combinations of routes cattle can take to slaughter. In the U.S., as of July 2007, there was an estimated over 104 million cattle located on more than 1 million premises (USDA/APHIS, 2007c). Due to the increased concentration and operational efficiencies recently attained by beef and dairy industries, operators must increasingly rely on interstate movement of their cattle to achieve their objectives (USDA/APHIS, 2011h). The extensive movements of animals between premises can require investigatory activities in multiple U.S. States and Canada (USDA/APHIS, 2008b). When animal health officials find a herd of origin that is determined to be infected (an index herd), the tracing of animal into and out of the index herd can be extensive identify hundreds to thousands of cattle that may have been exposed and moved to multiple other premise(s) (USDA/APHIS, 2008b). An example of the epidemiological investigation process and extent of dispersion of animals and (potential) dispersion of disease can be found in Appendix A.

Length of time to conduct investigations

When bovine TB infected cattle are found at the time of inspection at the slaughterhouse, they are traced to the herd of origin as quickly as possible under the current system. Anderson (1959b) stressed the importance of tracing cattle as rapidly as possible to minimize the spread of disease. When an investigation lacks information for a seamless trace to a herd of origin, all of the consignors (sellers) who contributed cattle to the slaughter lot (or feedlot) that contained the bovine TB lesioned animal must have their herds tested. If one of the consignor's herds is determined to be infected then that herd is presumed to be the herd of origin of the lesioned bovine detected at slaughter. Wagner (1988) explained that this type of scenario has taken one to two years or more to locate the herd of origin. The USDA has been discussing the need to improve trace-back capabilities and decrease the duration of investigations for some time. In November and December 2007, the USDA released "Advancing Animal Disease Traceability, Overview

and Synopsis" and "A Business Plan for Advancing Animal Disease Traceability", respectively, in which they stated the average time spent conducting a trace-back for the most recent 27 bovine TB investigations was 199 days; 125 days for the last 4 investigations (USDA/APHIS, 2007c). Forty-eight hours is stated as the USDA's optimum trace-back capability and work is being conducted to improve the animal disease traceability infrastructure in the U.S. to reach that goal (USDA/APHIS, 2007c).

The practice of raising dairy heifers in large dry lot facilities with feeder cattle

Another complication related to the U.S.'s ability to trace cattle in disease investigations is the practice of raising dairy heifers in large dry lot facilities with feeder cattle. These dry lot facilities have been known to bring in unidentified cattle in large numbers from multiple sources in multiple States (USAHA, 2009b).

Identification of herds with advance stages of disease

Early discovery of the disease is of prime importance in a disease eradication program (Ranney, 1970); however, the very nature of the slaughter surveillance process induces a constraint to controlling bovine TB in that the disease may not be detected until cattle within the herd reach advanced stages of disease. Routine testing has an advantage over slaughter surveillance in this regard as evidence has suggested that routine testing can locate herds with early stages of infection (Ranney, 1967). In the 60's, Ranney showed that bovine TB infection found in herds via trace-back were more often advanced disease, in comparison to, bovine TB found in herds by routine area testing, which were more often in early stages of disease (Ranney, 1970). Figure 3 shows the status of bovine TB infection when 70 herds were first found in 1969. Sixty-four percent of 25 herds found through routine testing were in early stages of infection and thirty-six percent of the herds were in advanced stages of infection. In contrast, forty-two percent of 45 herds found via trace-back investigations were early stages of infection and fifty-eight percent of the herds were in advanced stages of infection.
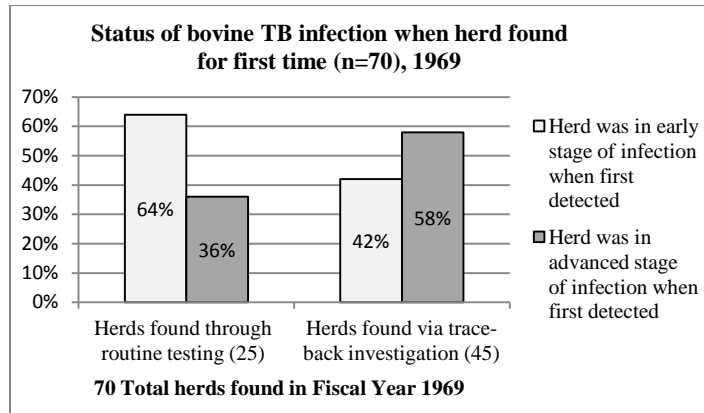
Figure 3: Status of bovine TB infection when herd found for first time (n=70), 1969

(Source: Ranney, 1970)

Figure 4 shows the status of bovine TB infection when 568 herds were first found during years 1964 to 1969. Seventy-four percent of 311 herds found through routine testing were in early stages of infection and twenty-six percent of the herds were in advanced stages of infection. In contrast, forty-nine percent of 257 herds found via trace-back investigations were early stages of infection and fifty-one percent of the herds were in advanced stages of infection. Serious concerns still exist regarding the possible lateness associated with detecting bovine TB infected herds through slaughter surveillance (Meyer, 1988).
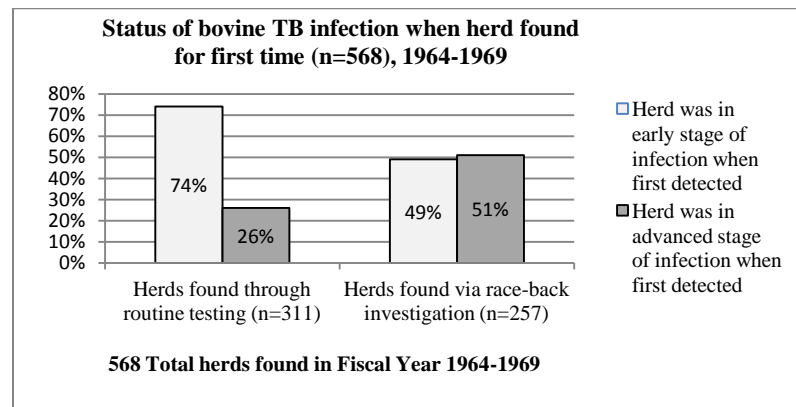
Figure 4: Status of bovine TB infection when herd found for first time (n=568), 1964-1969

(Source: Ranney, 1970)

1.3.8.2 Limitations of storage and reporting of slaughter surveillance and epidemiological case information

Since the U.S. eradication program began in 1917, an immense volume of data has been generated about infected animals, herds, and their resulting epidemiological investigations. Roswurm and Ranney (1973) pointed out that concentrating on current outbreaks only, without revisiting past activities, will insure the survival of the disease. In order to revisit past activities and summarize trends with the program a database was needed to handle bovine TB program information. Dr. Lonnie King of APHIS expressed the need for one in 1980: "Even though a subject is not traced to its herd of origin, information can often be gathered on market patterns, feedlots, cattle traders involved, etc., and many names will be generated. If these names would be stored in a dynamic database, that could be continuously updated, cross-referencing would be possible so that the names, markets, etc., that were involved in previous 6-35 trace-backs, would be flagged and printed as output." In 1984, a report from the members of the subcommittee to evaluate information management needs, training and research at the National Tuberculosis Eradication Program Evaluation Conference recommended that program records, program data and reports be computerized (USDA, 1985). By 1986, the bovine TB Program Planning and Support Staff for APHIS determined that a nation-wide microcomputer based information system should be developed to accurately process, retrieve, and analyze the enormous volumes of data, records, and reports (used to manage the bovine TB eradication program) and that the use of a nationwide system would standardize the procedures and processes used in the various states (Meyer, 1988).

In 1988, Dr. Robert Meyer completed his Master Thesis entitled: "Development of a Database Management System for the Bovine Tuberculosis Eradication Program." He explained in his thesis that much of the bovine TB program information, was held only in the minds of program people and had been lost as they have retired. Additionally, the data that existed was virtually unavailable because it was not readily accessible; the data were unorganized and handled in such a way that could it not be easily referenced, severely limiting the capability of enhancing the disease eradication program. Consequently, program personnel, whose time and availability was often already compromised, tended to concentrate more on recent herd outbreaks and, unfortunately, had less time to retrospectively investigate factors that

allowed bovine TB to persist (Meyer, 1988). He went on to explain that singular attempts had been made by a few states to better organize particular portions of the data, but for the most part, bovine TB data processing had been done by manual methods. Program documents were forwarded to program records personnel in each state who manually tabulated quarterly statistical reports; the reports were then forwarded to the national program support staff who tabulated a cumulative report for all states. Once these reports were submitted, the original documents were manually filed and later archived (Meyer, 1988). Requests for certain descriptive epidemiological reports, potentially useful in evaluating certain significant aspects of the program, were extremely difficult if not impossible to obtain in 1988. The costs incurred in personnel alone to manually search through existing records and compile a report were prohibitive even though such information would enhance the eradication program.

Dr. Meyer stated that it was not realistic to believe that improved funding or increased personnel to more effectively handle herd investigations would occur in the foreseeable future; consequently, in order to make further gains, better utilization of the tools now available was required. Additionally, better organization and use of the collected data could be an effective tool in the eradication effort (Meyer, 1988). The benefits of a dedicated computer-based system for the collection, organization, and analysis of field data were the focus of Meyer's work. He facilitated the creation of an ORACLE relational database for the program in 1988. The database was named the Tuberculosis Information Management System, more commonly referred to as the TIMS database. The purpose of the database was to (Meyer, 1988):

> 1) track post mortem examination and tuberculin test data,
>
> 2) schedule and track events related to a herd,
>
> 3) track epidemiological investigations of animals entering or leaving infected herds,
>
> 4) schedule retests of herds determined to be at high risk of acquiring tuberculosis,
>
> 5) incorporate animal population at risk data for each study population, and
>
> 6) produce descriptive epidemiological reports that would allow for better evaluation of program progress.

The database consisted of 15 data entry screens designed to collect data recorded on 16 official forms; 18 initial reports were developed to provide program managers and tuberculosis epidemiologists with more

timely information regarding the program. However, use of the TIMS database was discontinued in 1999 with the implementation of the Laboratory Information Management System (LIMS) system at National Veterinary Services Laboratory and the Generic Database (GDB) at the National headquarters (Schoenbaum, 2000). A result of its discontinuation, it was forecasted that it will be much more difficult to study surveillance trends for bovine TB in the future (Schoenbaum, 2000). In addition to the change in computer systems, it is probable that the database fell out of favor due to the intensive data entry requirements and was not deemed as pertinent for reporting purposes with less infected cattle found per year in recent years. Today, tuberculin test data are stored in a State's GDB, in a national reports database via the Automated Web-Based Data Submission Process (AWBDS) process, in annual state "Accredited Area Surveillance for Tuberculosis" (VS 6-38) reports, in the Emergency Management Response System (EMRS) in outbreak investigations, and in some cases, individual State recordkeeping systems (USDA/APHIS/VS, 2009c). VS' "Analysis of bovine TB Surveillance in Accredited Free States" mentions some of the complexity of data analysis, in that of the States queried for CFT test data, five States did not have data in the GDB and lack of access prevented queries in two AF States; however, the data were obtained for these two States from the AWBDS database and VS 6-35 Reports. Six States and the Virgin Islands had no approved testing data in AWBDS (USDA/APHIS/VS, 2009c).

All literature reviewed for this study highlight the complexities and challenges faced in eradicating the last traces of bovine TB in the U.S. and make evident the need of an assessment of the efficiency of tracing tuberculous cattle detected at slaughter back to the herd of origin.

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Chapter 2 – Material and Methods

Data Sources

Data used for this study were provided by the USDA/APHIS/Veterinary Services (VS) and consisted of a

bovine TB slaughter surveillance spreadsheet, containing information on all bovine TB lesions found

during the period 2001-2010. The related epidemiological investigation case files were also available for

evaluation. The data set contains information on all lesions found at slaughter that were submitted to the

National Veterinary Services Laboratories (NVSL) to be further evaluated and confirmed to be bovine TB

by means of histopathology, polymerase chain reaction (PCR), and culture and were confirmed to be histo-

compatible for Mycobacteriosis. A total of 480 lesions (tissue samples) were submitted to the laboratory

for the 2001-2010 time period. The slaughter surveillance summary spreadsheet included the following

data and bovine TB case information:

- Laboratory related factors: National Veterinary Services Laboratories (NVSL) accession number,
  histopathology results (compatible for bovine TB), polymerase chain reaction (PCR) results,
  culture results,

- Animal factors: slaughter date, age, sex, animal identification (ID), country or state of origin,
  most recent owner,

- Investigation related factors: fiscal year, investigation status / comments, case open or closed,
  case closure date, days from slaughter to closure,

- Slaughter plant factors: State primary (the State where the slaughter establishment was located or
  the State that has been identified as the original source of the cattle if different than the slaughter
  establishment state), State secondary (became the slaughter establishment State if cattle came from
  a State outside the slaughter establishment State), slaughter establishment name (removed for
  confidentiality), inspector name (removed for confidentiality), eligible for award, award
  comments, award amount, inspector state.

Detailed information on the lesions and their respective epidemiological investigations could be found in

the individual case files. Prior to 2006, these individual case files were maintained in paper form; since

2006, the case files have been maintained in an electronic format and/or in paper form. Excel[2] and

STATA[3] were used for data manipulation and analyses, as well as, STATA[4] for univariable analyses.

Inclusion criteria

Animals (bovines) with confirmed bovine TB were included in this analysis. For the purposes of this

study, a bovine TB case was considered confirmed if the submitted gross lesion was histocompatible for

Mycobacteriosis, PCR was positive for *Mycobacterium tuberculosis* complex and/or the culture results

were identified as *M. bovis*. Eighty one percent (n=389) of the 480 lesions submitted to the laboratory were

classified as bovine TB using histocompatibility for Mycobacteriosis, PCR for *Mycobacterium tuberculosis*

complex and/or culture results of *M. bovis*. From these 389 bovine TB positive lesions, 386 were disclosed

by bovines and three by cervids during the study period. From these 386, the majority of lesions, 347

(90%), were disclosed by fed cattle, and only 39 (10%) were disclosed by culled adult (beef and dairy)

cattle. Figure 5 summarizes the distribution of disclosed bovine TB lesions during the period 2001-2010.

The cervid bovine TB cases (n=3) were excluded from the analysis.

A "successful trace-back" was considered only for animals with a herd of origin potentially in the U.S.

For animals imported into the U.S., trace-back success was not considered because these investigations are

sent to the animal's home country for further investigation. VS had determined the country of origin for

the majority of cattle disclosing a bovine TB lesion at slaughter. The determination was made based on the

animal identification present at slaughter, if available, and/or by epidemiological investigation conducted

subsequent to the discovery of the bovine TB lesion. Cattle were classified as Mexican, Canadian, U.S., or

unknown. VS' criteria for a bovine being of Mexican origin was the presence of Mexican animal

identification tag and/or indication of Mexican origin based on the conducted epidemiological

investigation. VS' criteria for a bovine being of Canadian origin was the presence of Canadian animal

identification and/or indication of Canadian origin based on the conducted epidemiological investigation.

For imported animals from Mexico and Canada, although not the focus of this study, we assessed the

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[2] *Microsoft: 2007, Redmond, Washington*
[3] *Stata Statistical Software: Release 11.2, College Station, Texas*
[4] *Stata Statistical Software: Release 11.2, College Station, Texas*

feedlot premises where these animals resided in the U.S. prior to slaughter and quantified the number of lesions from each feedlot in Appendix C.
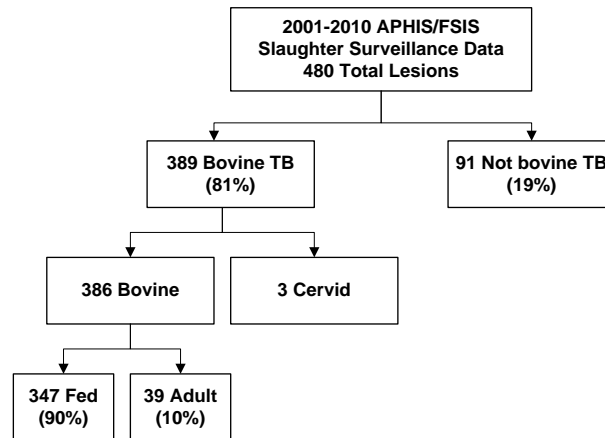
Figure 5: Distribution of submitted gross lesions, 2001-2010

Outcome definition

Successful trace-back to a herd of origin (objective 2):

The term "herd of origin" for a bovine TB case was defined as any livestock grouped together in a traditional herd, either the birth herd, most recent herd of residency (before slaughter), herd of primary residence or interim herd of residence in the U.S. "Herd of origin" did not include livestock in feedlots, backgrounding facilities, calf-raising facilities and livestock owned by livestock dealers; these facilities and circumstances are for congregating animals and were not considered a herd. A "successful trace-back" was recorded each time the "herd of origin" was identified by epidemiological investigation (outcome=1). Otherwise, if the "herd of origin" was not identified an "unsuccessful trace-back" was recorded (outcome=0).

Affected herd found (objective 3):

An "affected herd found" was recorded each time at least one infected herd was found in the U.S. as a result of a successful trace-back investigation that found additional bovine TB infected animals in the herd(s). Finding at least one additional bovine TB infected animal is required to confirm bovine TB in the "herd of origin" through a combination of tuberculin skin testing using CFT followed by CCT, gamma

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interferon (GI) assay, PCR and/or culture. Additional affected herds may be confirmed (identified through secondary trace-in or trace-out investigations) using previously described testing protocols (outcome=1). Otherwise, if testing did not confirm at least one affected herd(s), the outcome, for objective 2, was recorded =0.

Analysis

Descriptive statistics were performed in which the proportion of bovine TB cases that had a successful trace-back to the herd of origin and the proportion of these trace-backs that led to finding bovine TB infected ("affected") herds were calculated for objective 2 and 3, respectively. Paper and electronic files for the 386 cases disclosing a bovine TB lesion at slaughter were assessed independently and in collaboration with the VS to complete the stated objectives. The descriptive statistics were completed based on the age category of the bovine TB lesioned animals: fed and adult. Fed animals were less than or equal to two years of age and included castrated rodeo/roping cattle of all ages. Adult animals were sexually intact animals greater than two years of age. The trace-back success was determined by review of the case closing reports, miscellaneous case notes, the affected herd spreadsheet and discussion with VS personnel. The affected herd status was determined by review of the tuberculin test record reports, NVSL laboratory reports, case closing reports, miscellaneous case notes, the affected herd spreadsheet and discussion with VS personnel.

Initially, univariable analysis using parametric Chi-squared test and non-parametric Fisher's exact 2-sided test (cross tabulation, 2x2 tables) were conducted (for objective 4) to assess and compare the risk of a successful trace-back between different categories of selected factors. This approach was repeated in order to assess and compare the risk of finding an affected herd among different levels of selected factors. Factors evaluated for their potential association with the risk of a successful trace-back and the risk of finding affected herd(s), included:

> 1) Animal factors: official identification (ID), management (mgt) identification (ID), age (adult, female), gender (male, female), animal type (cow, bull, heifer, steer), home-bred versus purchased, residency period (in the seller herd for those animals that were purchased), breed,

2) Herd management factors: type of herd (beef, dairy), herd size, rodeo/roping cattle, and

3) Epidemiological investigation related factors: length of time to perform epidemiological trace-back investigations.

Official animal identification (ID) provides unique, permanent (or semi-permanent) and state specific identification of animals. For the purpose of this analysis official ID included[5]: 1) National Uniform Eartagging System (NUES) (bright or brite) tags, 2) brucellosis vaccination (Bang's) tags, and 3) USDA backtags. Management animal identification (or bangle tags) provides unique identification of animals; however, it is not permanent or state specific. Factors, which were not specifically listed in the slaughter surveillance spreadsheet, were identified by review of the tuberculin test record reports, case closing reports, miscellaneous case notes, the affected herd spreadsheet and discussion with VS personnel and were populated in the spreadsheet in order to be analyzed. These factors included type of herd, herd size, rodeo/timed event cattle, and length of epidemiological investigations.

Analyses by age, gender, and type of ID of the bovine TB infected animal(s) were conducted to assess the association between the presence of type of animal ID and the probability of achieving a successful trace-back to the herd of origin of a bovine TB case detected at slaughter.

Finally, a multivariable logistic regression model was developed (for objective 4) to test the hypothesis that selected risk factors were not associated with the risk of the outcome (trace-back success, failure) while controlling for the effect of other factors in the model.

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[5]*There were no Animal Identification Number (AIN) tags for the bovine TB lesioned animals detected at slaughter between 2001-2010.*

3.1 Success of trace-back investigation to herd of origin in the U.S. for fed and adult cattle disclosing a bovine TB lesion at slaughter during 2001-2010

A total of 386 cases of bovine TB in cattle were identified and confirmed during the study period. From these confirmed bovine TB cases, 347 (90%) were from fed (young) cattle and 39 (10%) were from adult cattle. The distribution (frequency and %) of successful trace-backs to a herd of origin for fed and adult cattle disclosing a bovine TB lesion at slaughter for 2001-2010 is shown in Figure 6.
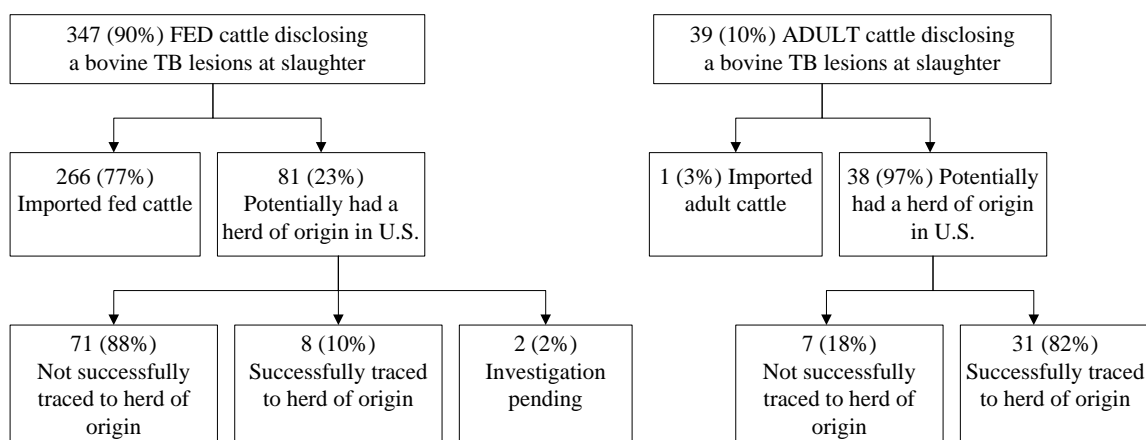
Figure 6: Distribution (frequency and %) of successful trace-backs to a herd of origin in the U.S. for fed and adult cattle disclosing a bovine TB lesion at slaughter, 2001-2010

Trace-back success for fed cattle

Of the 347 fed cattle in Figure 3 that disclosed a bovine TB lesion at slaughter, 266 (77%) were classified as imported animals (264 Mexican and 2 Canadian). Knowledge of their importation status was based on information available at slaughter or as a result of the epidemiological investigation. Trace-back to a "herd of origin" was not applicable to these animals. Out of the remaining 81 (23%) fed cattle with bovine TB lesions, only 8 (10%) were successfully traced back to a herd of origin in the U.S., 2 (2%) were still under investigation (pending) at completion of our analysis (May 2011), and 71 (88%) were not successfully traced-back to a herd of origin in the U.S.

Trace-back success for adult cattle

Of 39 adult cattle in Figure 3 that disclosed a bovine TB lesion at slaughter, 1 (3%) was classified as an imported animal (1 Canadian). From the remaining 38 (97%) adult cattle with bovine TB lesions, 31 (82%) were successfully traced back to a herd of origin in the U.S. and 7 (18%) were not successfully traced back to a herd of origin in the U.S.

Overall trace-back success

The overall (fed and adult combined) percentage of success in tracing bovine TB slaughter cases back to the herd of origin was 33% ((8+31)/(71+8+7+31)) for cattle that potentially had a herd of origin in the U.S.

3.2 Affected herd found in the U.S. through testing after a successful trace-back was achieved

Animal health officials were able to successfully trace-back a total of 39 cattle (8 fed and 31 adult), that disclosed a bovine TB lesion at slaughter during the 2001-2010 time period to a herd of origin in the U.S. The distribution (frequency and %) of successful trace-backs that yielded at least one affected herd (additional bovine TB infected animals) in the U.S. for fed and adult cattle disclosing a bovine TB lesion at slaughter for 2001-2010 is shown in Figure 7.
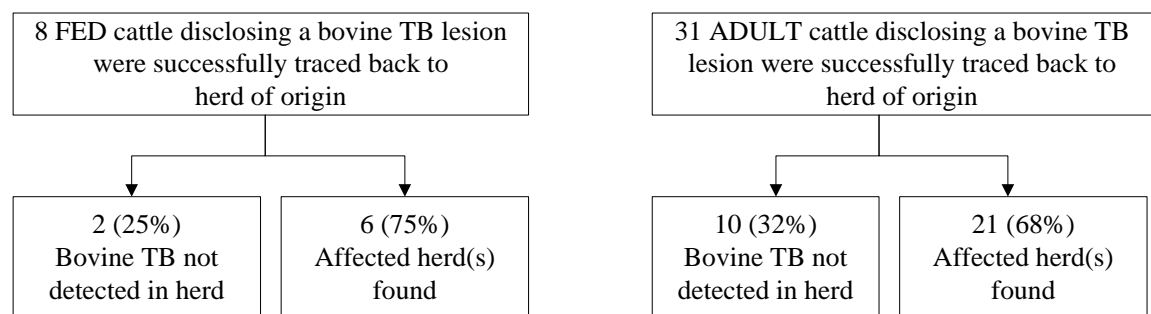
Figure 7: The number (%) of successful trace-backs that yielded at least one affected herd in the U.S. for fed and adult cattle disclosing a bovine TB lesion at slaughter, 2001-2010

Affected herd(s) found from fed cattle successfully traced-back

Figure 7 shows that from the 8 fed cattle disclosing a lesion at slaughter that were successfully traced to a herd of origin in the U.S., 6 (75%) yielded at least one affected herd when either the herd of origin or another herd (identified through secondary trace-in or trace-out investigations) was tested using the official

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bovine TB program tests. Two (25%) of the 8, did not detect bovine TB when the herd of origin or associated herds were tested.

Affected herd(s) found from adult cattle successful traced-back

Figure 7 shows that from the 31 adult cattle disclosing a lesion at slaughter that were successfully traced to a herd of origin in the U.S., 21 (68%) yielded at least one affected herd when the herd(s) was tested and 10 (32%) did not. Thus, additional bovine TB infected cattle were confirmed within at least 21 (68%) herds of successfully traced adult bovine TB lesioned cattle.

Overall affected herd(s) found from cattle successful traced-back

The overall (fed and adult combined) percentage of affected herd(s) found from successful trace-back investigations was 69% ((6+21)/(8+31)) for cattle that potentially had a herd of origin in the U.S.

3.3 Association of selected factors with the probability of a successful trace-back and identification of affected herds in the U.S.

Table 9 shows the frequency of the select animal factors (official ID, management ID, age and gender) and herd management factor (rodeo/roping) for bovine TB lesioned cattle that potentially had a herd of origin in the U.S., the proportion of these cattle that yielded a successful trace-back to a herd of origin in the U.S., the odds ratio (OR), probability value (p-value) and 95% confidence interval for the odds ratio (95% OR CI).

Table 9: Univariable analysis and odds ratios, of trace-back success for selected animal and herd management factors for cattle with a herd of origin potentially in the U.S.

Factor		Trace-back success		Proportion of trace-back success	Odds Ratio	p-value	95% OR CI
		Yes	No				
Official ID[a]	Present	25	4	86%	33.04	<0.001	10.26, 104.96
	Absent	14	74	16%	Ref		
Management ID[a]	Present	13	54	19%	0.22	<0.001	0.10, 0.50
	Absent	26	24	52%	Ref		
Age[a]	Adult	31	7	82%	39.30	<0.001	13.29, 116.27
	Fed	8	71	10%	Ref		
Gender[b]	Male	9	65	12%	0.06	<0.001	0.02, 0.16
	Female	29	13	69%	Ref		
Rodeo/Roping[a]	Yes	3	22[c]	12%	0.21	0.02	0.06, 0.72
	No	36	56	39%	Ref		

[a]Trace-back success was pending for two of the 119 cases with a herd of origin potentially in the U.S.
[b]Trace-back success was pending for two cases and gender was unknown for 1 case, of the 119 cases with a herd of origin potentially in the U.S.
[c]Eighteen rodeo/roping cattle were group together at market; all were part of one outbreak scenario

Animal factors

Official identification:

Table 9 shows the frequency of official identification (ID) for bovine TB lesioned cattle that potentially had a herd of origin in the U.S. and the proportion of these animals that yielded a successful trace-back to a herd of origin in the U.S. Of the bovine TB lesioned cattle detected at slaughter that potentially had a herd of origin in the U.S., only 25 percent (or (25+4)/117)) had official ID present at the time of slaughter. Table 9 shows that there was a statistically significant difference (p<0.001) with a higher proportion of successful trace-backs to a herd of origin in the U.S. for bovine TB lesioned cattle with official ID (86%). Table 9 also shows that the odds of a successful trace-back in cattle with official ID were 33.04 times higher than the odds in cattle without official ID (95% CI 10.26, 104.96).

Among the bovine TB cases in which a herd of origin was successfully found, no difference was found (at the 5% level of significance) between the proportion of affected herds found for cattle with official ID compared to cattle without official ID. Additionally, there were no statistically significant differences in

the proportion of affected herds found for cattle with any of the other select factors (mgt ID, age and gender) versus without the select factor.

Management identification:

The frequency of management identification for bovine TB lesioned cattle (that had a herd of origin potentially in the U.S.) and the proportion of these animals that yielded a successful trace-back to a herd of origin in the U.S. is shown in Table 9. Fifty-seven percent (or (13+54)/117)) of the bovine TB lesioned cattle detected at slaughter (that potentially had a herd of origin in the U.S.) had management ID. There was a lower proportion of successful trace-backs to a herd of origin in the U.S. for bovine TB lesioned cattle with management ID (19%) compared to bovine TB lesioned cattle without management ID (52%) and the difference was statistically significant (p<0.001)(shown in Table 9). The odds of a successful trace-back in cattle with management ID were 0.22 times lower than the odds in cattle without management ID (95% CI 0.10, 0.50)(shown in Table 9).

Age:

Among bovine TB lesioned cattle detected at slaughter that potentially had a herd of origin in the U.S., the majority (68%) were fed (young) cattle (or (71+8)/117) and the minority (32%) were adult cattle (or (31+7)/117). There was a significant difference (p<0.001) with a higher proportion of successful trace-backs to a herd of origin in the U.S. for adult bovine TB lesioned cattle (82%) compared to fed bovine TB lesioned cattle (10%). The odds of a successful trace-back among adult cattle was 39.30 times higher than the odds among fed cattle (95% CI 13.29, 116.27)(shown in Table 9). An additional analysis by age was done to assess the impact of official and management animal identification among different age categories (see Table 11and 14).

Gender:

The majority (64%) of the bovine TB lesioned cattle detected at slaughter that potentially had a herd of origin in the U.S. were male (or (9+65)/116) and the minority (36%) were female (or (29+13)/116). There was a significant difference (p<0.001) with a higher proportion of successful trace-backs to a herd of origin

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in the U.S. for female bovine TB lesioned cattle (69%) compared to male bovine TB lesioned cattle (12%). The odds of a successful trace-back among male cattle was 0.06 times the odds among female cattle (95% CI 0.02, 0.16)(shown in Table 9). An additional analysis by gender was done to assess the impact of official and management animal identification among different age categories (see Table 12 and 15).

Herd management factor

Roping/rodeo cattle:

Table 9 shows the frequency of bovine TB lesioned cattle that were rodeo/roping (time event) cattle and the proportion of these animals that yielded a successful trace-back to a herd of origin in the U.S. Among bovine TB lesioned cattle (detected at slaughter that potentially had a herd of origin in the U.S.), 21% (or (3+22)/117) were roping/rodeo cattle. There was a lower proportion of successful trace-backs to a herd of origin in the U.S. for rodeo bovine TB lesioned cattle (12%) compared to non-rodeo bovine TB lesioned cattle (39%) and this difference was statistically significant (p=0.02)(see Table 9). The odds of a successful trace-back investigation in roping cattle was 0.21 times the odds in non-roping cattle (95% CI 0.06, 0.72)(see Table 9).

Age by gender:

Table 10 shows the number of fed and adult cattle by gender for cattle with a herd of origin potentially in the U.S. The majority of fed (young) bovine TB lesioned cattle detected at slaughter were male (70/79 or 89%) while the majority of adult were female (33/37 or 89%).

Table 10: Count of fed and adult cattle by gender for cattle with a herd of origin potentially in the U.S.

	Gender		
	Male	**Female**	**Total**
Fed	70 (89%)	9 (11%)	79
Adult	4 (11%)	33 (89%)	37
Total	**74**	**42**	**116***

*For the 116 out of 119 cattle that were available for analysis.

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Association between official identification and trace-back success by age, gender and management

identification of the bovine TB lesioned animals

Official ID by age

To assess the association between official ID and trace-back success by age of the bovine TB lesioned cattle with a herd of origin potentially in the U.S. Table 11 was created.

Fed bovine TB cases:

Among the fed bovine TB lesioned cattle detected at slaughter with a herd of origin potentially in the U.S., only 4 percent (or 3/79) had official ID present at the time of slaughter. There was a significant difference (p<0.001) with a higher proportion of successful trace-backs to a herd of origin in the U.S. for fed bovine TB lesioned cattle with official ID (100%) compared to fed bovine TB lesioned cattle without official ID (7%). The odds of a successful trace-back among fed cattle with official ID compared to the odds among fed cattle without official ID was unable to be calculated since there were no fed cattle with official ID that were not successfully traced back.

Adult bovine TB cases:

In contrast, the majority (68% or 26/38) of the adult bovine TB lesioned cattle detected at slaughter (with a herd of origin potentially in the U.S.) had official ID present at the time of slaughter. There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for adult bovine TB lesioned cattle with official ID (85%) compared to adult bovine TB lesioned cattle without official ID (75%); however, this difference was not statistically significant (p=0.66, OR 1.83 (95% CI (0.38, 9.09)).

Table 11: Presence of official identification and association with trace-back success by age for cattle with a herd of origin potentially in the U.S.

	FED Official ID			ADULT Official ID		
	Present	**Absent**	**Total**	**Present**	**Absent**	**Total**
Trace-back successful	3	5	8	22	9	31
Trace-back not successful	0	71	71	4	3	7
Total	**3**	**76**	**79***	**26**	**12**	**38**
Trace-back proportion	100%	7%	10%	85%	75%	82%

*The trace-back success was pending for two cases; therefore, only 79 of the 81 fed animals with a herd of origin potentially in the U.S. were available for analysis.

Official ID by gender

Table 12 was performed to assess the association between official ID and trace-back success by gender of the bovine TB lesioned cattle with a herd of origin potentially in the U.S.

Male bovine TB cases:

In regards to gender, only 8 percent (or 6/74) of the male bovine TB lesioned cattle detected at slaughter (with a herd of origin potentially in the U.S.) had official ID present at the time of slaughter. There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for male bovine TB lesioned cattle with official ID (83%) compared to male bovine TB lesioned cattle without official ID (6%) and this difference was statistically significant ($p<0.001$). The odds of a successful trace-back among male cattle with official ID was 80.00 times higher than the odds among male cattle without official ID (95% CI 9.26, 854.06).

Female bovine TB cases:

In contrast, 52 percent (or 22/42) of the female bovine TB lesioned cattle detected at slaughter (with a herd of origin potentially in the U.S.), had official ID present at the time of slaughter. There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for female bovine TB lesioned cattle with official ID (86%) compared to female bovine TB lesioned cattle without official ID (50%) and this difference was statistically significant ($p=0.02$, OR 6.33 (95% CI 1.49, 26.32)).

Table 12: Presence of official identification and association with trace-back success by gender for cattle with a herd of origin potentially in the U.S.

| | MALE | | | FEMALE | | |
| | Official ID | | | Official ID | | |
	Present	Absent	Total	Present	Absent	Total
Trace-back successful	5	4	9	19	10	29
Trace-back not successful	1	64	65	3	10	13
Total	**6**	**68**	**74***	**22**	**20**	**42***
Trace-back proportion	83%	6%	12%	86%	50%	69%

*The trace-back success was pending for two cases and gender was unknown for one case; therefore, only 116 (74 male and 42 female) of the 119 animals with a herd of origin potentially in the U.S. were available for analysis.

Official ID by management ID

To assess the association between official ID and trace-back success by management ID of the bovine TB lesioned cattle with a herd of origin potentially in the U.S. Table 13 was created.

Management ID present:

Ten percent (or 7/67) of the bovine TB lesioned cattle detected at slaughter (with a herd of origin potentially in the U.S.) had management and official ID present at the time of slaughter. There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for bovine TB lesioned cattle with management and official ID (86%) compared to bovine TB lesioned cattle with management ID and without official ID (12%) and this difference was statistically significant (p<0.001). The odds of a successful trace-back among cattle with both forms of ID was 45.43 times higher than the odds among cattle with management ID and without official ID (95% CI 5.93, 437.03).

Management ID absent:

Forty-four percent (or 22/50) of the bovine TB lesioned cattle detected at slaughter (with a herd of origin potentially in the U.S.) had official ID without management ID at the time of slaughter. There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for bovine TB lesioned cattle without management and with official ID (86%) compared to bovine TB lesioned cattle with neither management ID nor official ID (25%) and this difference was statistically significant (p<0.001, OR 19.00 (95% CI 4.48, 78.91)).

Table 13: Presence of official identification and association with trace-back success by management identification for cattle with a herd of origin potentially in the U.S.

| | Management ID present | | | Management ID absent | | |
| | Official ID | | | Official ID | | |
	Present	Absent	Total	Present	Absent	Total
Trace-back successful	6	7	13	19	7	26
Trace-back not successful	1	53	54	3	21	24
Total	**7**	**60**	**67***	**22**	**28**	**50***
Trace-back proportion	86%	12%	19%	86%	25%	52%

*The trace-back success was pending for two cases; therefore, only 117 of the 119 animals with a herd of origin potentially in the U.S. were available for analysis.

Association between management identification and trace-back success by age, gender, and official identification of the bovine TB lesioned animals

Management ID by age

To assess the association between management ID and trace-back success by age for bovine TB lesioned cattle with a herd of origin potentially in the U.S., Table 14 was created.

Fed bovine TB cases:

Management ID was present at slaughter on 72 percent (or 57/79) of the fed bovine TB lesioned cattle that potentially had a herd of origin in the U.S. There was a lower proportion of successful trace-backs to a herd of origin in the U.S. for fed bovine TB lesioned cattle with management ID (7%) compared to fed bovine TB lesioned cattle without management ID (18%); however, this difference was not statistically significant (p=0.21, OR 0.34 (95% CI 0.08, 1.38)).

Adult bovine TB cases:

For the adult bovine TB lesioned cattle with a herd of origin potentially in the U.S. 26 percent (or 10/38) had management ID present at the time of slaughter.   There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for adult bovine TB lesioned cattle with management ID (90%) compared to adult bovine TB lesioned cattle without management ID (79%); however, this difference was not statistically significant (p=0.65, OR 2.45 (95% CI -7.03, 11.95)).

Table 14: Presence of management animal identification and association with trace-back success by age for cattle with a herd of origin potentially in the U.S.

| | FED | | | ADULT | | |
| | Management ID | | | Management ID | | |
	Present	Absent	Total	Present	Absent	Total
Trace-back successful	4	4	8	9	22	31
Trace-back not successful	53	18	71	1	6	7
Total	**57**	**22**	**79***	**10**	**28**	**38**
Trace-back proportion	7%	18%	10%	90%	79%	82%

*The trace-back success was pending for two cases; therefore, only 79 of the 81 fed animals with a herd of origin potentially in the U.S. were available for analysis.

Management ID by gender

Table 15 was created to assess the association between management ID and trace-back success by gender for bovine TB lesioned cattle with a herd of origin potentially in the U.S.

Male bovine TB cases:

In regards to gender, 68 percent (or 50/74) of the male bovine TB lesioned cattle (that potentially had a herd of origin in the U.S.) had management ID present at the time of slaughter. There was a lower proportion of successful trace-backs to a herd of origin in the U.S. for male bovine TB lesioned cattle with management ID (6%) compared to male bovine TB lesioned cattle without management ID (25%) and this difference was borderline statistically significant (p=0.05). The odds of a successful trace-back among male cattle with management ID was 0.19 times lower than the odds among male cattle without management ID (95% CI 0.05, 0.79).

Female bovine TB cases:

For the female bovine TB lesioned cattle (that potentially had a herd of origin in the U.S.) 40 percent (or 17/42) had management ID present at the time of slaughter. There was a lower proportion of successful trace-backs to a herd of origin in the U.S. for female bovine TB lesioned cattle with management ID (59%) compared to female bovine TB lesioned cattle without management ID (76%); however, this difference was not statistically significant (p=0.24, OR 0.45 (95% CI 0.12, 1.65)).

Table 15: Presence of management identification and association with trace-back success by gender for cattle with a herd of origin potentially in the U.S.

	MALE Management ID			FEMALE Management ID		
	Present	**Absent**	**Total**	**Present**	**Absent**	**Total**
Trace-back successful	3	6	9	10	19	29
Trace-back not successful	47	18	65	7	6	13
Total	**50**	**24**	**74***	**17**	**25**	**42***
Trace-back proportion	6%	25%	12%	59%	76%	69%

*The trace-back success was pending for two cases and sex was unknown for one case; therefore, only 116 (74 male and 42 female) of the 119 animals with a herd of origin potentially in the U.S. were available for analysis.

Management ID by official ID

To assess the association between management ID and trace-back success by official ID of the bovine TB lesioned cattle with a herd of origin potentially in the U.S. Table 16 was created.

Official ID present:

Twenty-four percent (or 7/29) of the bovine TB lesioned cattle detected at slaughter (with a herd of origin potentially in the U.S.) had management and official ID present at the time of slaughter. There was the same proportion of successful trace-backs to a herd of origin in the U.S. for bovine TB lesioned cattle with official and management ID (86%) compared to bovine TB lesioned cattle with official ID and without management ID (86%); therefore, there was no statistically significant difference (p=1.00, OR 0.95 (95% CI 0.11, 11.02)).

Official ID absent:

Sixty-eight percent (or 60/88) of the bovine TB lesioned cattle detected at slaughter (with a herd of origin potentially in the U.S.) without official ID had management ID present at the time of slaughter. There was a lower proportion of successful trace-backs to a herd of origin in the U.S. for bovine TB lesioned cattle without official and with management ID (12%) compared to bovine TB lesioned cattle with neither form of ID (25%); however, this difference was not statistically significant (p=0.13, OR 0.40 (95% CI 0.13, 1.22).

Table 16: Presence of management identification and association with trace-back success by official identification for cattle with a herd of origin potentially in the U.S.

	Official ID present			Official ID absent		
	Management ID			**Management ID**		
	Present	**Absent**	**Total**	**Present**	**Absent**	**Total**
Trace-back successful	6	19	25	7	7	14
Trace-back not successful	1	3	4	53	21	74
Total	**7**	**22**	**29***	**60**	**28**	**88***
Trace-back proportion	86%	86%	86%	12%	25%	16%

*The trace-back success was pending for two cases; therefore, only 117 of the 119 animals with a herd of origin potentially in the U.S. were available for analysis.

Table 19 in Appendix E shows the frequency of the presence of any ID (both official and mgt ID, official ID only, mgt ID only) versus neither forms of ID for bovine TB lesioned cattle that had a herd of origin potentially in the U.S. and univariable analysis of trace-back success. Table 20 and Table 21 in Appendix E show the association between the presence of any ID (both official and mgt ID, official ID only, mgt ID only) versus neither forms of ID and trace-back success by age and gender, respectively for bovine TB lesioned cattle with a herd of origin potentially in the U.S.

Summary of animal ID status and trace-back success for bovine TB lesioned cattle with a herd of origin potentially in the U.S.:

Table 17 was created to analyze the animal identification status and trace-back success of cattle disclosing lesions at slaughter that potentially had a herd in the U.S.

Table 17: Presence of animal identification and trace-back success for cattle disclosing lesions at slaughter with a herd of origin potentially in the U.S.

Official identification present at slaughter? (NUES tag, brucellosis vaccination tag, and/or USDA back tag)	Management (tag) identification present at slaughter? (i.e. Yellow 135 or 407)	Trace-back successful?	Number (%) of infected cattle
Yes	Yes	Yes	6 (5%)
Yes	No	Yes	19 (16%)
No	Yes	Yes	7 (6%)
No	No	Yes	7 (6%)
Yes	Yes	No	1 (1%)
Yes	No	No	3 (3%)
No	Yes	No	53 (45%)
No	No	No	21 (18%)
	Total		**117 (100%)**

Of the 117 bovine TB lesioned cattle with a herd of origin potentially in the U.S., 39 cattle were successfully traced back. Five percent (or 6/117) had both forms of ID and were succesfully traced back, 16% (19/117) had official ID only (no management ID) and were successfully traced back, 6% (7/117) had management ID only (no official ID) and were successfully traced back. These results indicated that the presence of either kind identification or simultaneous use of both may faciliate trace-back success. Contrary to what might be expected, 6% (7/117) had neither form of ID and were successfully traced back indicating that trace-back investigations were conducted successfully without identification. Review of the epidemiological trace-back investigation case files for these cases indicated success was the result of various things such as complete and accurate receipts and records about the individual animal for all parties involved, the use of the live weight, gender, breed and color to faciliate tracing, the investigation was a direct trace from slaughter back to the owner (there were minimal steps from herd of origin to slaughter), genotyping facilitated the investigation (the slaughtered animal had a strain of *M. bovis* that was previously indentified in an infected herd and records indicated that the animal was from the same area), perseverance of personnel invovled with the investigation, and/or all producers that had cattle penned with the infected animal at slaughter were tuberculin tested, one producer was found to have additional infected animals in their herd; consequently, that herd was determined to be the herd of origin.

Of the 117 bovine TB lesioned cattle with a herd of origin potentially in the U.S., 78 cattle were not successfully traced back. One percent (1/117) had both forms of ID present at slaughter and were not

successfully traced back, 3% (3/117) had official ID (but no management ID) and were not successfully traced back and 45% (53/117) had a management ID (but no official ID) and were not successfully traced back. These results show that the presence of animal ID (either kind or both) does not ensure trace-back success. Management ID by itself in particular does not ensure trace-back capability. Reasons for not being able to successfully trace cattle that had ID at slaughter included: insufficient record-keeping by owners made trace-back to herd of origin impossible, an official ID tag was issues twice in a ten year period, and/or the ID did not faciliate tracing beyond the feedlot or calf-raiser. Eighteen percent (21/117) did not have either forms of ID present at slaughter and none of them were successfully traced back. These cases indicate that a lack of animal identification may hinder trace-back efforts.

Epidemiological investigation related factor

Length of time to perform epidemiological investigations (the time required to trace-back and locate the herd of origin (affected or non-affected) :

This analysis could only be performed for epidemiological investigations on bovine TB cases where a successful trace-back to a herd of origin was achieved and a herd was tested. Information was available only for 28 bovine TB cases. The mean length of the 28 analyzed epidemiological investigations was 61.4 days (S.D. = 72.3). The median length of the 28 analyzed epidemiological investigations was 39.5 days (min = 7 days, Q1 = 15 days, Q3 = 75.5 days, max = 335 days). Appendix G provides a detailed description of this analysis.

Multivariable logistic regression model

A multivariable model was built to assess the effect of each factor on the outcome (trace-back success, failure) while controlling for the other factors in the model. Factors that showed an association with trace-back success (p-value < 0.25) were used to build the multivariable model. Official ID, management ID, age and gender were included. The final model shown in Table 18 included official ID and age (variables < 0.05).

Table 18: Multivariable logistic regression analysis to evaluate associations between the presence of animal identification and age on the likelihood of successfully tracing bovine TB cases to herd of origin

Trace-back success	Odds Ratio	Std. Error	P-value	95% Conf. Interval
Official ID (yes, no)	7.06	5.20	0.008	1.66, 29.93
Age (adult, fed)	15.47	9.79	<0.001	4.47, 53.48

The model showed that when controlling for age (regardless of age), the odds of successful trace-back were 7.06 times greater for cattle with official ID than without official ID (95% OR CI (1.66, 29.93), p-value =0.008). Additionally, when controlling for official ID (regardless of the presence of official ID), the odds of successful trace-back were 15.47 times greater for adult than for fed cattle (95% OR CI (4.47, 53.48), p-value<0.001). Interaction was assessed for official ID and age; however, no significant interaction was found.

Chapter 4 – Discussion

For approximately the last one hundred years, bovine TB has presented a special challenge to the U.S. regarding its control and eradication. Significant accomplishments were undoubtedly achieved in the U.S. in the early stages of State-Federal Cooperative bovine TB control program. The prevalence of the disease was reduced to less than 0.11% at the animal level (number of reactors) by the 1950's (Anderson, 1959b); however, today the goal of eradication remains elusive (USDA/APHIS/VS, 2009b). The U.S. has access to the strategies and tools that have eradicated bovine TB in other countries, however; eliminating the last traces of the disease in the U.S. has been exceedingly difficult and is taking longer than anticipated.

Between the period 2001-2010, 386 bovine TB cases were confirmed in the U.S. through slaughter surveillance. These cases were identified by meat inspectors at slaughter and subsequently confirmed by laboratory techniques. Bovine TB continues to be detected sporadically around the country and remains a threat to the livelihood and prosperity of U.S. livestock producers and to the reputation and trade of U.S. dairy and beef products. According to the California Department of Food and Agriculture, nine States detected bovine TB in herds between 1998 and 2010 (Merlo, 2010). Although bovine TB is no longer considered a major public health threat in the U.S. today, *M. bovis* can still be a human health hazard for people who work in close contact with cattle and for people who choose to consume unpasteurized products (Danker *et al.*, 1993 and CDC, 2005).

Slaughter surveillance plays a crucial role in the U.S. bovine TB control program because it is the primary tool for identifying bovine TB cases. The identification of granulomatous lesions and subsequent confirmation of the lesions compatible for Mycobacteriosis is what triggers epidemiological investigations. The aim of the epidemiological investigations conducted by USDA veterinary officials is to identify the "source" (herd) of the bovine TB lesioned animal and to identify other herds and/or animals at risk of being exposed to *M. bovis* in an effort to control the spread of the disease within the national herd. Concerns have been expressed regarding to the ability and efficiency of the current system to find the source(s) of the cattle disclosing bovine TB lesions at slaughter and identifying associated animals infected with *M. bovis* (Anderson, 1959b; Ranney, 1970; Kaneene *et al.*, 2006; USDA/APHIS, 2007c; USDA/APHIS/VS, 2009b

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and 2009d; USDA/APHIS, 2011e). Additionally, in recent years, awareness of food safety issues has increased and the capability of tracing food, including meat (cattle) from "farm to the fork" has become increasingly important due to a number of food-borne related illnesses and deaths experienced in the U.S. (Golan *et al.*, 2004). "Farm-to-fork" refers to the act of tracing food through all stages of production: harvest, storage, processing, packaging, sales and consumption (Edelstein, 2009). Virtually all meat products sold in the U.S. are traceable from retailer(s) back to the slaughter plant(s) (or processor(s)); however, a comparable level of traceability is not applicable when tracing cattle from the slaughter establishment back to the farm (Golan *et al.*, 2004).

Our study results confirm the concerns expressed with regards to animal traceability in the U.S. as the overall (fed and adult combined) percentage of success in tracing bovine TB cases identified at slaughter back to the herd of origin was 33% for the period 2001-2010 (out of 117 cases that potentially had a herd of origin in the U.S.). This figure is much lower than the 50-70% success "rate" cited by Kaneene *et al.* (2006) and supports the view(s) that currently in the U.S. there is a need to review and improve cattle traceability capabilities.   In comparison, other countries have reported much higher percentages of success in tracing bovine TB cases identified at slaughter back to the herd of origin. For example, Mexico reported the following percentages of success in tracing bovine TB cases from slaughter back to the herd of origin: 80.48% for 2009 (from 1103 cases detected), 89.55% for 2010 (421 cases detected), 90.29% for 2011 (505 cases detected) (Reyes, 2011). In the Republic of Ireland, in a particular study evaluating the risk of bovine TB at the herd level after a bovine TB lesion was identified at slaughter during 2003, all 1,713 bovine TB cases identified at slaughter were successfully traced back to the herd of origin (Olea-Popelka *et al.*, 2008). In the Republic of Ireland, this level of success can be achieved as the animal identification and management system rules require that animals are first cleared for slaughter on the computer system before they can be accepted for slaughter, and this process involves a supplier herd number being provided and the system validating that the animal is actually recorded as part of that herd (O'Keeffe, pers. comm.).

Of the approximate 34 million head slaughtered per year in the U.S., relatively few bovine TB cases are detected each year. We found that on average 39 bovine TB cases are found per year, of which 27 are

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imported and 12 are non-imported potentially having a herd of origin in the U.S. (shown in Appendix D, Figure 10). However, due to the chronic nature of the disease, a few bovine TB lesions detected at slaughter have the potential to present a problem when the majority of the bovine TB cases (66% or 78 of 117) are not successfully traced back to the herd of origin, as our study indicates. The low proportion of successful trace-back investigations may eventually lead to a re-emergence of the disease and represents a significant impediment to the goal of eradication. Thus, the U.S. should maximize efforts to successfully trace-back all the bovine TB cases found at slaughter in order to prevent sources of infection to remain undetected among the national herd.

With regards to the age profiling of bovine TB cases in the U.S., the USDA classify animals as "fed" or "adult". These two classes of animals are deemed to pose different epidemiological risks in the bovine TB control program. Fed cattle are defined by the program as less than or equal to two years of age and include castrated roping cattle of all ages. Adult cattle are greater than two years of age and sexually intact. Fed cattle, with their short lifespan of 24 months or less, are perceived to present minimal risk for spreading infection to other animals, particularly to domestic breeding cattle. Fed cattle of domestic origin are primarily only exposed to breeding cattle at their originating cow-calf operation. Once fed cattle leave a cow-calf operation, they are deemed to not be associated with an established herd and reside with cattle that will all be shipped to slaughter within several months (Golan *et al*., 2004). Imported fed cattle are also perceived to present minimal risk for spreading infection because like domestic fed cattle they do not have an established herd after being imported and ideally do not have the opportunity to mix with the domestic breeding cattle. The majority of cattle slaughtered in the U.S. are fed steers and heifers and the minority are culled dairy cows and culled beef cows and bulls (Golan *et al*., 2004). From the 386 bovine TB lesions disclosed at slaughter during 2001-2010, the majority (90%) of the lesions (347 bovine TB lesions) were disclosed by fed cattle and only 10% (39) were disclosed by adult cattle.

From the (347) *fed* cattle that disclosed lesions, the majority (266 or 77%) were from cattle that were determined to be imported and 81 (or 23%) were from cattle that potentially had a herd of origin in the U.S.

Of the 81 fed cattle that potentially had a herd of origin in the U.S., State and Federal animal health officials only were able to successfully trace 10% (8 out of 81) back to the herd of origin; for various reasons, a large percentage (88% or 71 out of 81) were unable to be successfully traced back to a herd of origin and 2% (2 out of 81) of the epidemiological investigations were pending at the time of completion of this analysis.   Fed animals (potentially born in the U.S.) that were not successfully traced back may have (predominately) acquired infection in feedlots prior to their final destination (slaughter) posing little risk to national herd; however, there is a possibility that these fed cattle acquired the infection at a cow-calf operation, stocker or back grounding operation prior to feedlot. It is extremely important to successfully trace each infected fed animals back to each of their locations prior to slaughter, in particular back to the cow-calf operation where breeding cattle reside. Of the 950,000 (total) cattle operations in the U.S. in 2009, 753,000 (79%) were cow-calf operations (USDA/APHIS/VS, 2011h). Generally, domestic fed cattle take one of the three routes below to slaughter (Golan *et al*., 2004)(see Figure 5 in Appendix B) and may spend 30-60 days in a preconditioning (back grounding operation) between production stages (Blezinger, 2005):

> 1) cow-calf operation direct to slaughter,
>
> 2) cow-calf operation, stocker (calf raiser / grower), feedlot, slaughter, or
>
> 3) cow-calf, feedlot, slaughter.

If a calf was infected at a cow-calf operation and the slaughter initiated trace-back investigation was not successful at identifying the operation, additional domestic animals may be infected at the operation and may continue to spread the disease unnoticed until additional animals in the herd reach advanced stages of disease and are detected by slaughter surveillance. An additional concern with not tracing back to cow-calf operations, is the fact that the grazing lands (pasture, range land, Federal land) that domestic cows and growing calves are reared on may be adjacent and not separated by fencing, meaning that animals belonging to different owners may comingle and get mixed (Golan *et al*., 2004). Thus, despite the relatively lower risk posed by fed cattle (compared to adult cattle) it is extremely important to maximize efforts to successfully identify the herd of origin for fed bovine TB cases. If epidemiological investigations successfully trace domestic fed calves to all of their locations prior to slaughter the bovine TB program would be able to:

1) ensure that *M. bovis* does not persist on any of the associated locations, particularly cow-calf operations,

2) ensure exposed animals (and herds) are identified, tested, and reactors to the skin test are removed,

    a) ensure exposed animals that resided at feedlots and were not destined for slaughter are tested (e.g. heifers raised in feedlots),

3) ensure facilities (cow-calf operations, stocker operations, backgrounding facilities and feedlots) are quarantined and managed according to protocol, and

4) verify that any exposed animals that were destined for slaughter from feedlots or other facilities were not diverted from slaughter (e.g. diverted into rodeo careers)

Even though the focus of this thesis does not pertain to the imported bovine TB infected cattle, a brief discussion of them is justified. Of the 266 lesions disclosed by imported fed cattle during 2001-2010, almost all of them (264 or 99.2%) were determined to have originated from Mexico. These results show that, despite an interim rule being published in the U.S. Federal Register (66 FR 20187-20190, Docket No. 00-102-1) in 2001 that amended animal import regulations to better ensure imported cattle were free of bovine TB, infected Mexican cattle are still entering the U.S. The rule required:

1) an import permit be obtained for the importation of cattle from Mexico,

2) information regarding each premise where cattle intended for export to the U.S. have resided, and

3) a certificate regarding the tuberculosis history of the herds from which a group of cattle is assembled for export to the U.S. The certificate of health must:

    a) be issued by a veterinary officer of the government or a veterinarian accredited by the government of the region of origin, and

    b) state that all cattle imported into the U.S. be tested for tuberculosis based on the status of their state of origin in Mexico, except for cattle imported for immediate slaughter and for cattle imported from Canada.

The rule also added a definition for herd of origin and revised the definitions of herd, official tuberculin test, and whole herd test. With approximately 1 million (live) cattle imported every year into the U.S. from Mexico (Peel *et al*., 2011), it is important to note that the required testing before importation does not conclusively ensure that these animals are disease free upon entry. Diseased animals may falsely test negative to the CFT test due to its lack of sensitivity or when the test is not used properly. An example of how infected animals enter the U.S. was provided at the 115th Annual USAHA meeting in Buffalo, NY in October 2011 and described instances of young animals being group together and tested as a herd in compliance with the USDA's definition of a herd. According to CFR title 9, Section 77.2 a herd is defined as any group of livestock maintained for at least 4 months on common ground for any purpose (excluding livestock assembled at feedlots), or two or more groups of livestock under common ownership or supervision, geographically separated but that have an interchange or movement of livestock without regard to health status, as determined by the Administrator. Testing young animals as a herd, who may be incubating the disease, can lead to the possibility that they will all test negative and the certificate regarding the bovine TB history of the herd will incorrectly state that they are healthy. Knowing that pre-import testing does not ensure the importation of healthy animals; the USDA should consider implementing additional methods to reduce the probability of bovine TB infected animals entering this country. Methods could include quarantined feedlots or herds in the U.S. for Mexican animals not destined for slaughter, required tuberculin testing of all Mexican imported cattle (not destined for slaughter) regardless of the status of the Mexican state of origin, and/or a revised definition of a herd. If the definition of a herd was revised to include breeding animals and/or the time requirement that livestock must be maintained on common ground was lengthen, then the health certificate of the imported animal's herd would more accurately reflect the true disease status of the animals within the herd.

Upon entry into the U.S., imported fed cattle typically follow one of three routes to slaughter (Golan *et al*., 2004)(see Figure 8 in Appendix B):

> 1) pasture, feedlot, slaughter,
>
> 2) feedlot, slaughter, or
>
> 3) direct to slaughter (after importation).

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Bovine TB infected fed cattle from Mexico are deemed to be of low risk of infecting domestic animals, if when on pasture, they are kept away from domestic animals, and, when in feedlot, they go straight to slaughter; however, epidemiological investigation case files imply this was not always the case for the 2001-2010 infected Mexican cattle. At the time of our study, the true extent of interaction between Mexican fed cattle and U.S. fed and adult cattle appears to be unknown and undocumented. Once Mexican cattle pass the importation requirements and enter into the U.S., there are no (further) regulations applied to these animals. Mexican animals can graze on pasture (before entering the feedlot) where they may have direct or fence-line contact with domestic animals. Additionally, infected Mexican cattle may contaminate soil that is shared with domestic cattle. Available evidence has shown that the duration of infectivity of *M. bovis* from environmental sources, such as pasture, urine and feces, for a susceptible species such as cattle, is measured in weeks (Morris *et al.*, 1994). With the capability to remain viable on soil and in feces for weeks at a time depending on the environmental conditions, the transmission of *M. bovis* is exceptionally difficult to control. Knowing that the practice of sharing of pasture among imported and domestic cattle is a potential pathway for transmission of the bovine TB, policy makers and producers should attempt to reduce this risk.

In the feedlot, Mexican cattle may have direct or indirect contact with non-terminal U.S. cattle (i.e. replacement heifers raised in feedlots). The practice of having non-terminal U.S. cattle in feedlots that may have contact with Mexican cattle presents a high risk to the national herd as U.S. cattle could get exposed to *M. bovis* in a feedlot before going back to a U.S. established herd. Moreover, anecdotal evidence has indicated that Mexican cattle destined for slaughter have been diverted from slaughter for participation in rodeo events. In order to reduce the risk of infection between imported and domestic cattle, these encounters and practices should be prevented. We recommend the USDA investigate the risk associated with Mexican cattle on pasture(s) and in feedlot(s) and determine how much interaction they are truly having with U.S. fed and adult cattle.

To help prevent interaction and disease transmission between Mexican and domestic cattle, USAHA resolution No. 50 (2008) recommended that USDA/APHIS/VS require steers and spayed heifers originating

from Mexican states or zones which have never historically achieved Accredited-Free Status only be

allowed importation into the U.S. if transported directly from the port of entry or first point of assembly to

feedlots, pastures and pens which do not contain (or will be used by) breeding domestic cattle. I

recommend that changes to regulations and policies governing the administration of the bovine TB

program address this resolution.

For this study, the success of trace-back investigations was not applicable to the subset of imported cattle

because these animals do not have established herds once imported and these investigations are reported to

the exporting country's animal health officials in order to trace to the animal's birth herd and all premises

prior to exportation (when they are a part of a more traditional herd). Animal health officials in the U.S.

did however attempt to trace imported cattle to all of their locations between importation and slaughter.

These locations included pasture land and/or feedlot. Appendix C, Figure 9, shows the number of infected

Mexican cattle per feedlot during the 2001-2010 time period. Continuing to assess the number of infected

Mexican cattle per feedlot could be beneficial for the U.S. bovine TB control and eradication program.

From all (386) bovine TB lesions identified in 2001-2010, thirty-nine (10%) were disclosed by adult cattle.

According to USDA officials, bovine TB infected adult cattle pose the most risk to the health of the

national herd. Domestic adult cattle have a longer lifespan than fed cattle due to their role in being part of a

breeding herd and a higher probability of contact (direct or indirect) with other animals throughout their

lifespan. Thirty-eight of the thirty-nine adults that disclosed lesions had a herd of origin potentially in the

U.S. The success "rate" of tracing back to the herd of origin (for these lesioned adults) was 82% (31 out of

38). This high rate (82%) is anticipated because U.S. officials prioritize the identification of the herd of

origin of adult bovine TB cases. The rate is commendable because the adults pose the most risk to the

health of the national herd. The lack of success in identifying the herd of origin for 7 (18%) adult animals

that potentially had a herd of origin in the U.S. hinders U.S. bovine TB control efforts.

Based on reviewing the case files for the bovine TB lesions detected at slaughter, the reasons for (fed and adult) cattle not being successfully traced back to a herd of origin included:

1) irreconcilable, incomplete, and/or illegible industry (producer, dealer/broker, market, feedlot, slaughter plant) receipts, records and documentation, and/or

2) absent, insufficient or incorrectly correlated animal identification.

When animal identification was not present at slaughter, traces were conducted based on the animal's hot (live) weight, as well as, color and breed, if available. Tracing an animal based on their hot weight, color and/or breed is not efficient. Management identification was found to facilitate tracing-back at times but was largely insufficient at successfully tracing back because it did not facilitate tracing cattle between premises. Animal identification incorrectly associated with carcasses at slaughter complicated and lengthened some investigations.

The results of our third objective bring to fruition the impact of not finding the herd of origin for the 7 adults (and associated exposed animals) and 71 fed cattle disclosing bovine TB lesions at slaughter. After a successful trace-back was achieved, our results indicate that the majority of times tuberculin tests were performed on identified herds, infected animals were identified in 69% (overall) of the epidemiological investigations of these herds (or 75% (6 out of 8 cases) for the fed and 68% (or 21 out of 31 cases) for adult bovine TB cases successfully traced back). This finding indicates that failure in finding the herd of origin for bovine TB cases is a significant constrain in controlling bovine TB in the U.S. Not finding herd(s) of origin that are likely to be infected means infected animals will remain undetected in the herds and infection will persist and spread within the national herd. For the epidemiological investigations that were not successful in finding the herd of origin, with the high probability that infection will be found, we can only speculate:

1) the number of herds to which infected animals may have been sold to (inadvertently spreading the infection),

2) the number of months or years before infected herds may be discovered through slaughter surveillance,

3) the number of years this may delay the eradication of bovine TB from the states involved,

4) the degree of hazard to human health for the individuals with direct livestock contact,

5) the financial loss to the livestock owners whose herds may become infected, and

6) the tax dollars that must be added to the program as a result of the potential spread of infection. Ranney (1970) stated the exact concerns with regards to an outstanding bovine TB trace-back investigation in 1965-66. These concerns need not be speculative issues and could be remediated through implementation of a robust system for tracking cattle (i.e. a fully functional animal identification system) that enables a quick response when bovine TB lesioned cattle are detected at slaughter.

The 69% overall risk of finding additional infected animals after a herd of origin is found and all animals are tested, differs greatly from the scenario in the Republic of Ireland, where once a bovine TB lesion was detected at slaughter in 2003 and the herd of origin(s) were found and tested, only 20% (or 338 out of 1713) of herds disclosed additional reactors to the first herd test (using the single intradermal cervical comparative test)(Olea-Popelka *et al*., 2008). For the second herd test after a bovine TB lesion was found at slaughter, in total approximately 35% of herds in the Republic of Ireland disclosed additional reactors to the tuberculin test (Olea-Popelka, pers comm). Differences between the U.S. and the Republic of Ireland with respect to the probability of finding infected herds may be explained by differences in disease control management and the temporal component in the bovine TB testing regime. In the Republic of Ireland, herds are tested regularly, at least once a year, and bovine TB reactors are removed. By contrast, in the U.S., there is not a testing regime that allows constant and periodic removal of infected animals; only when a bovine TB lesion is identified at slaughter, an epidemiological investigation will start and if identified, additional bovine TB reactors will be removed from herds. Since in the U.S. infection on the farm is only detected as a result of a bovine TB lesion being identified at slaughter, the infection has had time to spread within and between herds from the time of onset in a herd until a bovine TB lesion is detectable at slaughter. The higher proportion of herds disclosing additional infected animals in U.S. herds (69% overall) after a bovine TB lesion was detected at slaughter in comparison to the Republic of Ireland (20%) could (among other factors) be due to the fact that the disease control management program in the U.S. may inadvertently allow the disease to spread between (and within) herds prior to being detected.

Thus, the results found while studying the U.S. scenario (the proportion of successful trace backs leading to identification of additional bovine TB reactor cattle) suggests that disease may be present within herds for a prolonged amount of time prior to being detected and may spread to a substantial number of other herds prior to being detected. With respect to the amount of infection that is found when herds in the U.S. are tested, a relatively high intra (or within) herd prevalence has been found in several epidemiological investigations that successfully traced back the bovine TB case found at slaughter to the herd of origin in the U.S. This suggests that the disease was present within the herds for a prolonged amount of time prior to being detected. For example, in March 2010 in Colorado, bovine TB lesions were identified in a cow during regular slaughter inspection. Successful trace-back to the herd of origin of the bovine TB lesioned cow and CFT testing of 498 adult cattle resulted in 162 suspects (32.5%) (the herd had 908 animals in total). Testing the suspects with the comparative cervical test resulted in 124 positive results (25% of the 498 adult cattle tested) (USAHA, 2010). Consequently, the herd was subsequently depopulated. Through post mortem examination, from the 908 total animals in the herd, 10.5% (95 animals) were culture positive for *M. bovis* from suspect lesion submitted to the laboratory (Francisco, per comm). The extent of animals testing positive to the CCT and the number of gross lesions found upon necropsy implies that the disease was present within the herd for a substantial length of time before being identified. Moreover, the dairy herd had a whole herd test (WHT) for bovine TB one year before the outbreak, in compliance with Colorado's requirement for dairies to have a WHT every 3 years. At the time of the WHT, there was one CCT reactor and one CCT suspect who were necropsied and no gross lesions were found. A variety of lymph nodes from different sites were submitted for histopathology and culture; however, no *M. bovis* was isolated (Francisco, per comm). Three possibilities may explain this scenario: 1) the CCT reactor and suspect were false positives and no infection was present in the herd at the time of the WHT, 2) the CCT reactor and suspect were false positives and the infection entered the herd sometime between the WHT and March 2010, or 3) the CCT reactor and suspect were true positives and a low prevalence of the disease was present in the herd as indicated by the WHT (one CCT reactor and one CCT suspect) and *M. bovis* was not cultured. The identification of bovine TB in this Colorado dairy led to hundreds of trace investigations and the identification of six other facilities positive for bovine TB (USAHA, 2010). This particular example

reflect the importance of detecting bovine TB as early as possible and the potential implications for a particular herd (and other herds) of missing the infection when present.

Similar scenarios of finding index herds and contact herds with high intra-herd prevalence have been reported in the Netherlands, a country considered to be free of bovine TB that also relies on slaughter surveillance as the primary method of detecting disease. Similar to the approach in the U.S., in the Netherlands if a potential bovine TB induced lesion is detected by carcass inspection, all animals in that particular herd are tuberculin tested intradermally. In addition, all cattle of source herds (trace-backs), herds that purchased animals (trace-outs), possible contact herds (fence-line contacts) and herds within a certain radius (buffer zone) are tuberculin tested intradermally. In the 1990's surveillance resulted in the detection of nine outbreaks in Dutch cattle herds where the primary infected herds (5 out of 9) had a high (tuberculination test) prevalence (up to 80%) upon detection. An outbreak in1999 resulted in detection of a Dutch dairy farm of which approximately 70% of the animals were test positive, followed by detection of 10 positive animals in 9 contact herds (van Asseldonk *et al.*, 2005; Fischer *et al.*, 2005). For the herds with substantial prevalence, it was concluded, under the Dutch epidemiological scenario, that infection may have been present in the affected herds for a long time without being detected (van Asseldonk *et al.*, 2005). It was estimated that after introduction of the infection into a herd, the median time until a detection of a bovine TB lesion was 302 weeks (approximately 5 years)(Fischer *et al.*, 2005). With such a substantial intra herd prevalence and extensive spread of detectable disease for the outbreak discovered in 1999, investigators questioned "whether the bovine TB spread within and between herds was detected quickly enough to prevent the risk of substantial disease-control costs and to guarantee food safety" and whether "the current surveillance system, visual inspection of carcasses at the slaughterhouse, is efficient enough to detect infected cattle in time and to maintain the official bovine TB-free status" (van Asseldonk *et al.*, 2005; Fischer *et al.*, 2005).

The results found in this study for the U.S. are in agreement with the reports from the Netherlands. Once a bovine TB lesion is found at slaughter and a herd of origin is identified, the results of the tuberculin test (or other test) applied to the herd may reflect exposure to *M. bovis* from several years ago. Both countries rely

on slaughter surveillance as the primary method of detecting infection because it is the most cost effective method for a country to implement with a low disease prevalence. The high proportion (69% overall) of U.S. epidemiological investigations identifying additional affected herds (after a lesion was identified at slaughter), is likely the result of the combination of the chronic nature of bovine TB and the deficit of routine and systematic testing and removal of reactors from herds. The reliance on slaughter surveillance is a double edged sword in that with its implementation, if and when disease is detected at slaughter and the herd of origin is identified, disease found within the herd has likely progressed to advanced stages and has had ample opportunity to spread between herds, thus resulting in relative high intra-herd prevalence and a high likelihood of finding additional affected herds. Therefore, under this epidemiological scenario, when a bovine TB lesion is detected at slaughter, the U.S. needs to maximize their ability to find the herd of origin as a means of finding additional infected animals (herds). Failure to identify the herd of origin for each and every cattle disclosing bovine TB lesions at slaughter will increase the likelihood of infection to remain undetected for years, thus increasing the possibility of spread within and between herds and posing a significant constrain to the control and eradication of bovine TB from the U.S.

A minority, of the bovine TB slaughter cases that were successfully traced back to a herd of origin in the U.S., identified no further bovine TB infected animals (herds) when tested. Twenty-five percent (or 2 out of 8) successfully traced fed cases did not find additional infection in the herd and 32% (or 10 out of 31) successfully traced adult cases did not find additional infection in the herd. It is of extreme importance to notice that not finding bovine TB infected animals while testing the identified herd of origin, does not necessarily mean that bovine TB is absent from the herd. Not finding infected animals upon performing a herd test could be due to the fact that:

- no disease is present within the herd (the disease was appropriately managed and removed, or all infected cattle were sold to other herds or sent to slaughter by the time the identified herd was tested),
- the lack of complete sensitivity of the program tests,
- the tests were not properly administered,

- the tests were plugged (deliberate interference where chemicals are used in the cattle's feed to make them unable to respond to the tests (Meyer, 1988)), or

- the incorrect herd of origin was identified.

Concerning the factors associated with the probability of a successful trace-back being achieved for bovine TB cases that potentially had a herd of origin in the U.S., first, it is necessary to clarify that forms of official animal identification considered for this study were brucellosis vaccination tags, National Uniform Eartagging System (NUES, referred to as brite tags), and back tags. These forms of animal ID should not be confused with official animal identification (i.e. animal passports cards) that are used in countries that have fully implemented (official) National Animal Identification Programs. These three forms of official ID used in the U.S. are not subject to the same requirements as is the case in countries that have mandatory animal identification systems that allow, in centralized manner, the tracking of animal movements from birth to slaughter by the use of a unique animal identifier. Our univariable analysis revealed that the percentage of success in tracing bovine TB slaughter cases back to the herd of origin was significantly different for animals with and without official identification (86% and 16%, respectively, p<0.001, OR 33.04). The presence of management identification proved to inhibit trace-back success with a lower proportion of successful trace-backs to a herd of origin for lesioned cattle with management ID (19%) compared to without management ID (52%) (p<0.001, OR=0.22). The univariable analysis between age and trace-back success and gender and trace-back success showed there was a significant difference (p<0.001, OR=39.30, and p<0.001, OR=0.06, respectively) in the success for tracing adults (82%) versus fed (10%) and male (12%) versus female (69%). However, caution should be used when directly interpreting these results. Upon further analysis we found that the distribution of official ID was remarkable higher among adult, female bovine TB cases compared to fed, male bovine TB cases.

With regard to age, a small percentage (only 4%) of the fed bovine TB cases had official ID at slaughter in comparison to the majority (68%) of adult bovine TB cases. Overall only 10% of fed cattle were successfully traced back to a herd of origin in comparison to the majority of adult cattle (82%). When looking at the distribution of official ID by age and the association with trace-back success, all (100%) of

the fed cattle with official ID were successfully traced back in comparison to only 7% without official ID. While 85% of the adults with official ID were successfully traced back versus 75% without, indicating that the USDA is able to successfully trace adult cattle that lack official ID. This was somewhat anticipated because the USDA places more emphasis on tracing adult animals due their increased risk of spreading infection and reflects the determination of the animal health officials to find the herd of origin for these animals, despite the absence of official ID. The unequal distribution of ID found among these fed and adult bovine TB cases suggests an imbalanced application of official ID as a result of industry practices.

In a similar manner, with regard to gender, only 8% of the male cattle that disclosed lesions had official ID in comparison to the majority (52%) of female cattle. Overall only 12% of male cattle were successfully traced back to a herd of origin in comparison to the majority of female cattle (69%). When looking at the distribution of official ID by gender and the association with trace-back success, the majority (83%) of the male cattle with official ID were successfully traced back to a herd of origin in comparison to only 6% without official ID. With respect to the female cattle, 86% with official ID were successfully traced back versus 50% without official ID. Once again, indicative of a possible industry practice (intentional or unintentional) that results in the imbalanced application of official ID. In addition to discovering the imbalance of official ID amongst fed, male lesioned cattle, we discovered that the majority of fed (72%) had management ID and the majority of male (68%) had management ID, possibly indicative of industry practice to use management ID on steers instead of official ID. It is important to note that the majority of fed lesioned cattle were male (89%).

The results of the multivariable logistic regression model indicates that when controlling for age, the odds of successful trace-back are 7.06 times greater for cattle with official ID than without official ID and these results were statistically significant (OR 95% CI: 1.66, 29.93, p-value =0.008). Thus, overall, this result suggest that the presence of official ID significantly increases the probability of a successfully tracing a bovine TB case identified at slaughter back to the herd of origin. Therefore, the increased application of official ID on all classes of cattle, specifically on fed, male cattle, would increase the probability of successfully tracing bovine TB cases back to a herd of origin. It is important to note that management ID

did not remain in the final model, indicating that management ID is not associated with trace-back success. When controlling for official ID, the odds of successful trace-back are 15.47 times greater for adult than for fed cattle (OR 95% CI; 4.47, 53.48, p-value<0.001). This result reflects the fact that USDA is giving more emphasis to epidemiological investigation on adult bovine TB cases due to the relative higher risk these animals pose and consequently they have been significantly more successful in finding the herd of origin for adult bovine TB cases, regardless of the presence of official ID.

Even though our results clearly indicate that the presence of official ID increases the likelihood of successfully identifying the herd of origin for bovine TB cases in the U.S., it is important to notice that the presence of official ID does not ensure success; exhibited by 4 cases where official ID was present but the trace was not successful. On the contrary, a number of bovine TB cases without official ID (14 cases) were successfully traced back. Seven of these cases had management ID, possibly indicating, that it can be helpful in tracing back depending on the scenario. These findings indicate that other factors (present during an epidemiological trace-back investigation, i.e. good receipts and records and/or management ID) played an important role in achieving a successful trace-back. In conclusion, official ID facilitated trace-back success for the majority of bovine TB cases that were successfully traced back; however, under the current bovine TB surveillance system in the U.S., it did not always ensure success.

Even when official ID was present, adequate receipts and records were needed to facilitate trace-back success. Upon review of the bovine TB case files, the success in tracing cattle without animal ID were the result of accurate and complete records and documentation from producers, dealers/brokers, livestock markets, and slaughter plants. This finding emphasizes the fact that trace-back success is highly dependent on the completeness (adequacy) of documentation and/or successful communication of all parties involved and reflects the dedicated efforts of the USDA officials. The results from this study indicate the highly variable and unpredictable nature of tracing bovine TB cases in the U.S. The variability (in success of tracing cattle to the herd of origin) could be reduced if methods were implemented to standardize trace-back investiagations.

Even though the focus of this thesis does not pertain to rodeo/roping bovine TB infected cattle, a brief discussion of them is justified. Thirty-one cattle that disclosed bovine TB lesions at slaughter were "rodeo" animals. Animals used for rodeo or roping events, typically steers, present their own epidemiological risk. Rodeo steers can travel extensively around the country and live into their adult years. If they are infected with *M. bovis*, they pose a risk of exposing other animals to infection. However, similar to fed cattle, rodeo steers typically do not have established herds. Ideally, they are kept separate from breeding animals and are sent to a feedlot prior to slaughter once their rodeo careers are over. For the purposes of this study, the majority of rodeo/roping animals (29 of the 31) were classified as fed animals; two were classified as adult in concordance with USDA/APHIS/VS. There was a lower percentage of trace-back success for rodeo/roping than non-rodeo/roping cattle (12% and 39%, respectively). These results indicate that rodeo cattle are more difficult to trace, possibly due to their extensive movement around the country and the intricacy of the rodeo industry. However, it is important to note that 18 of the 25 rodeo/roping cattle were all part of one specific bovine TB outbreak scenario (in 2008) where they were grouped together at market and none of them were successfully traced back.

Of extreme importance is to note that the results, from the multivariable (and univariable) analysis, described in our study do not incorporate the effort and time required by animal health officials to successfully identify the herd of origin for bovine TB cases in the U.S. Throughout the process of conducting this study, we found that the trace-back investigation process was consistently extremely labor and time intensive despite the success that state and federal animal health officials had tracing some cattle. The length of time to perform the epidemiological trace-back investigations could not be adequately assessed (incorporated in our analysis) due to insufficient information stored for most bovine TB cases epidemiological investigations; however, we estimated the length for those bovine TB cases for which data were available. For epidemiological reasons, we were interested in assessing the time it took to find a herd and test it with the tuberculin test after a lesion was detected at slaughter (rather than how long it took to complete a trace from an administrative perspective). Thus, for 28 bovine TB cases which available information, we calculated the number of days between the slaughter date in which the bovine TB lesion was identified and the first tuberculin test date and/or PCR positive date. We found that the average time

spent to conduct a trace-back investigation (to trace an animal from slaughter back to their herd of origin) was 61.4 days (SD = 72.3 days), median = 39.5 days, with a range from 7 to 335 days). These results show that the time required to successfully trace back herds in the U.S. and perform the first skin test is highly variable. As such, measures should be implemented in order to reduce this variability and explore the implementation of approaches to shorten the time required to complete epidemiological investigations, thus allowing the implementation of control methods that will reduce the spread of infection within and between herds in the U.S.

It was also found that the "case closure" date for the epidemiological investigations did not follow specific and/or standardized criteria. Thus, epidemiological investigations were closed under non-uniform parameters and the "case closure" date could not be relied upon to analyze how long it took to complete a bovine TB case investigation. As such, we recommend defining specific criteria for designating a bovine TB case investigation as "closed."

Since time plays such an important role in the epidemiology of bovine TB, we suggest that the USDA periodically assess the achievement of trace-back success and calculate, as done in this study, the length of time required to find (affected and non-affected) herds. Standard criteria should be used to calculate the length of epidemiological investigations with respect to the time required to find herds. Keeping an accurate record of the epidemiological investigation length will facilitate a more accurate and objective analysis of this factor and will allow USDA officials to critically evaluate the efficiency in completing epidemiological trace-back investigations in a timely manner. Early identification and removal of infected cattle is crucial to achieve the goals of the bovine TB program in the U.S. Our results, and hence our recommendations related to the time associated with a successful trace-back, are in agreement with the basic principle that early identification and removal of infected cattle is dependent on successfully tracing bovine TB infected cattle detected at slaughter back to the herd of origin as rapidly as possible (Anderson, 1959b). Further analysis with direct applications and implications for the USDA personnel could be done to compare how long it takes and how challenging it is to find the herd of origin for adult cattle with and

without the presence of official ID. However, to be able to do this and other similar analysis, accurate temporal data is needed.

The current bovine TB data format in the U.S. did not allow the evaluation of factors that have been found to be significantly associated with the probability of a successful trace-back and identification of bovine TB affected herds in other studies (O'Keefe, 1992; Griffin *et al*., 1996; O'Mairtin *et al*., 1998b; Collins, 2001; Martin *et al*., 1999; Hammond, 1999; O'Sullivan & O'Keefe, 1997; Goodchild & Clifton-Hadley, 2001; Olea-Popelka *et al*., 2004; Griffin *et al.*, 2005; Olea-Popelka *et al*., 2008). Under the current bovine TB slaughter surveillance process in the U.S., data on the following herd and bovine TB lesioned animal factors were incomplete, un-obtained and/or stored in a form not accessible for analysis:

- class of lesioned animal (cow, bull, heifer or steer),
- home-bred versus purchased nature of the lesioned animal,
- residency period in the herd,
- breed,
- type of herd (beef, dairy),
- herd size,
- geographical location of the farm,
- prevalence of bovine TB in the area, and
- bovine TB history of the herd.

Appendix H details the reasons why these factors could not be assessed in this study. These factors for which not enough quantity or quality of data were available or were "missing" are the focus of our recommendations for improvements in the current national surveillance system. Recommendations related to the collection, storage and analysis of slaughter surveillance data are presented in Appendix J.

While conducting the analysis described in this study, a number of challenges were encountered due to the nature of the current system for identifying cattle (animal identification forms), and the manner in which epidemiological investigation information is recorded. The first challenge was that there were complexities trying to determine the "country of origin" for bovine TB cases, and specifically determining whether or

not cattle were domestic (U.S.). The current data format did not provide a platform to make this determination in a straightforward manner. Hence, to make this determination, we used two available resources: animal ID if it was present at slaughter and/or the notes in the epidemiological investigation case files. It is important to know that at slaughter the only indication that a bovine TB case was of U.S. origin is the presence of animal ID, if available. Through the process of conducting the subsequent epidemiological trace-back investigation, animal health officials can confirm, in some situations, that the animal was in fact of U.S. origin. Thus, for this study, the following information and criteria (process of elimination) were used to determine whether or not a bovine TB case was a domestic cattle. Regarding forms of animal identification, bovine TB cases found at slaughter could have had the presence of:

    1) any one or a combination of U.S. official ID (brucellosis vaccination tag, USDA backtag,

    and/or NUES (brite) tag)(none of them had AIN tags),

    2) management identification which is not country or state specific,

    3) Mexican ID,

    4) Canadian ID, or

    5) no identification at all.

The epidemiological investigations processes indicated that cattle presenting lesions at slaughter that were imported more often than not had Mexican official ID (160 cases) or Canadian official ID (2 cases) (see Appendix F). Then, cattle that lacked ID (or only had management ID) were pursued with diligence by animal health officials during the epidemiological investigation and were often able to determine whether an animal was likely imported and reported them as such. For example, if a bovine TB lesioned animal was determined to have been in a slaughter pen with cattle owned by a consiginer that only deals with Mexican cattle, animal health officials could reasonable conclude that the bovine TB lesioned animal was Mexican (even though the animal lacked ID). Table 27 in Appendix F shows that 104 lesioned cattle that lacked official ID were determined to be "most likely" of Mexican origin by way of the epidemiological investigation process. For the purposes of this study, any cattle that were most likely of Mexican origin (104 cases) or Canadian (1 case) origin were classified as imported. Thus, the remaining 119 bovine TB cases (386 confirmed bovine TB cases – 267 determined to be Mexican or Canadian) were then determined to be non-imported, thus, potentially having a herd of origin in the U.S. Seventy-two (69 fed and 3 adult)

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of these 119 bovine TB cases did not have any animal identification present at slaughter and their country
of origin was not able to be determined during the epidemiological investigation. However, since the
epidemiological trace-back investigations conducted on these cattle did not find evidence to conclude these
animals were imported or "most likely imported", these animals were classified in our study as potentially
having a herd of origin in the U.S. This criterion allowed any bovine TB case with an indication of being
imported to be classified as such. This was a conservative measure taken to minimize misclassification
when determining if bovine TB cases were of U.S. origin. The number of animals without any form of
identification has decreased in recent years (shown in Appendix D, Figure 12).

The difficulty with using U.S. official forms of ID as means of identifying animals of U.S. origin is that the
forms of official ID, while indicative of nationality of the cattle, are not absolute proof of U.S. origin.
These official ID's currently used are not applied at birth, have the potential to be applied to non-U.S.
cattle, are not applied to all U.S. cattle, can be removed despite their intention to be permanent, and in fact
originally, were not designed to indicate an animal's country of origin[6]. The intentions and limitations of
each form of official ID are summarized as follows:

- Bright tags: This form of identification is used when official identification is required and is not
  specific to any particular disease program (USDA, 2011f). The tag may be applied at any given
  point in an animal's lifetime (they are necessarily not applied at birth) and are primarily applied to
  animals destined for interstate movement.
- Brucellosis vaccination tag (Bang's tags or Official Calfhood Vaccination (OCV) tags): This form
  of identification was required only for certain young, female cattle (aged 4 to 12 months) moving
  into and out of states designated as Class B or C for brucellosis (USDA/APHIS, 2011h; Cattle

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[6] *The AIN tag has a country code for the U.S. (840) but there are not requirements that the tag be applied
at birth; the tag is applied at the convenience of the producers. It is used in the same manner as the bright
tag; it is applied to meet all official ID requirements. There were no Animal Identification Number (AIN)
tags for the bovine TB lesioned animals detected at slaughter between 2001-2010.*

Today, 2011). Since all 50 states are currently brucellosis-free the tag is essentially voluntary (note: once vaccinated the ear tag and tattoo are required) and the number of cattle identified in this manner has steeply declined (USDA/APHIS, 2011h). When attempting to determine the country of origin, the advantage of the brucellosis vaccination tag over the other forms of official ID, is that the application of the tag takes place very close to birth, increasing the likelihood that the animal was from the U.S. The application of the tag early in the animal's life also facilitates tracing closer to the birth herd.

- USDA backtags (or Market Cattle Identification (MCI)): This tag, issued by APHIS, provides a temporary unique identification for each animal that it is applied too (USDA, 2011f). Other reports state that this form of identification was intended to provide a means of determining brucellosis status of animals and is placed solely on the shoulders of breeding beef and dairy cattle that go through a livestock market (Golan *et al*., 2004).

- Brands: Brands are considering official ID by the USDA; however, they were not considered official ID for the purposes of this study due to the fact that they are removed early in the slaughter process and are separated from the carcass. Consequently, they cannot be used to facilitate trace-back investigations related to bovine TB.

Other issues encountered with official ID discovered through the review of the investigation case files included: official ID's were issued twice and official ID was removed after the animal was purchased by a new owner (management practices were such that any existing ID was removed upon arrival and a new (different) tag was applied).

The issues described with the current forms of animal identification reveal the lack of overall uniformity in the current (U.S.) system. This lack of uniformity makes it difficult to identify cattle that are born and raised in the U.S. and affects the ability to trace bovine TB cattle. With variability being inherent in recordkeeping, animal identification should serve as a tool to streamline the identification of cattle and their herds; however, whether animal identification serves that purpose is contingent on how it is implemented. Revamping the animal identification system in the U.S. to become more uniform, consistent and comprehensive would simultaneously facilitate and expedite the identification of cattle, the tracing of

infected cattle to their herds of origin and all premises from birth to slaughter, thus allowing the testing of high risk animals (the identification of infected herds) and the effective and efficient control of bovine TB in the U.S. Additionally, it would allow the U.S. to collect more precise data, that when analyzed, would reveal more telling information regarding the disease. Despite the reduction over the last one hundred years in the disease prevalence of bovine TB, a reliable system for tracing cattle would be advantageous to address the remaining pockets of infection of bovine TB as well as other disease outbreaks should they occur.

Another challenge encountered while conducting this study was that determining whether a trace-back investigation was successful (or not) and whether at least one affected herd was found was not a straightforward task due to the manner in which the epidemiological investigation data was recorded and stored. With respect to the recording of epidemiological data, some of the issues encountered were as follows: the majority of the case information was hand written, the structure of the forms used to collect data left room for significant variability in the information recorded, inconsistent criteria was applied amongst animal health officials in regards to what made a case closed, untraceable, and successful versus non-successful and the data was not collected with this specific intention in mind. Often the outcome of interest was not clearly indicated and had to be concluded based on the other available information. As a result, a considerable amount of time and energy was spent evaluating case by case reading and interpreting the case notes to be able to determine the bovine TB cases that were successfully traced back to a herd of origin (versus those that were not) and determining whether at least one affected herd was found. If incomplete, ambiguous or contradictory case information was encountered, USDA TB program staff was consulted for clarification. In summary, it is important to mention that during this study, I dedicated considerable amount of time and efforts in verifying the information related to the main outcomes of interest using all existing resources at the time of this study.

After evaluating all available data, most (96% or 112/117) of the U.S. bovine TB cases in this study provided conclusive information with regards the main outcome of interest (successful trace back to the "herd of origin"). It is important to note that upon conducting this analysis, four fed bovine TB cases had

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incomplete (definitive) information concerning the "herd of origin" (detected at slaughter during 2005). At the time of our analysis, the record reviewed for these four bovine TB cases indicated that these animals were traced back to a "calf-rising" operation, which did not met our definition for a "herd of origin", hence the outcome of the epidemiological trace-back investigations for these cases were classified as unsuccessful. Additionally, one fed bovine TB case (detected at slaughter during 2004), was included in our analysis as successfully trace-back to the herd of origin and at least one affected herd found; however, under some ambiguity. The specific case numbers for these five animals are presented in Appendix K. I recommend further evaluation for these five fed bovine TB cases.

Additionally, due to the complexity of the current system in the U.S. and the resulting lack of uniformity with respect to data collected, challenges also were present when defining the term "herd of origin". Initially, it was defined as the herd in which an individual animal was born and raised or, in concordance with the standard criteria used by the USDA, as a group of livestock that was maintained on common ground for at least 4 months, except for livestock assembled at feedlots (CFR, 2010c). However, upon review of the epidemiological investigation files it was necessary to define the term ("herd of origin") as any livestock grouped together in a traditional herd setting, either as the birth herd, most recent herd of residency (before slaughter), herd of primary residence, or interim herd of residence in the U.S., and consequently, the "success" (in this study) was dependent on whether or not a herd was found that the bovine TB case resided in. The term "herd of origin" however, did not include livestock in feedlots and calf-raising facilities; these facilities for congregating cattle were not considered a "herd". If a different or stricter definition of a herd is desired in future studies, data collected by investigators on what herd (in the animals life cycle) was found would have to be more specific and structured in a manner that would facilitate detailed analysis.

Furthermore, under the current U.S. bovine TB surveillance system, it was not possible to evaluate the completeness and extent of the trace-back investigations as to whether the animal health officials found all of the previous locations where the bovine TB animal identified at slaughter resided, whether they fully exhausted all of the associated pathways where exposed animals may have gone (trace-outs) and whether

they fully exhausted the pathways in which infection may have entered a herd (trace-ins). Knowing this type of information would reveal strengths and weakens in the current U.S. system and the weakness could be remediated as modifications to the system are made. Based on the findings of this study, future research on the efficiency of the epidemiological investigations themselves is warranted.

Another characteristic and limitation of the current system identified during our analysis, was that the data did not allow us to quantify the exact number of affected herds resulting from each trace-back investigation initiated at slaughter. Initially, our third objective, was to count how many affected herds resulted from each individual trace-back investigation; however, the recordkeeping of the epidemiological trace-back investigations did not facilitate determining exactly how many different affected herds came from an individual trace. For example, one successful trace-back investigation could yield multiple affected herds and some trace-back investigations lead to the same affected herd. Nevertheless, we were able to quantify the number of trace-backs that found at least one bovine TB affected herd. It would be valuable for the bovine TB control program to be able to report how many affected herds came from each (slaughter initiated) trace-back investigation because it would indicate how widespread the disease is.

In summary, and regarding the complexities and challenges discussed while conducting this study, it is my opinion that a more expedite and straightforward analysis of bovine TB cases data could be achieved in the future if modifications to the current data collection and storage are implemented. Thus, in Appendix G, H, I and J, I provide a number of specific recommendations that would help the USDA to improve the recording and storage of bovine TB epidemiological case information that will facilitate future analyses.

It is important to note that for this study the analytical unit of interest was each bovine TB case and whether the associated epidemiological investigation was successful in finding the "herd of origin", or not. There were a few scenarios in our study where more than one bovine TB case traced back to the same herd of origin. Specifically two bovine TB cases lead back to the same herd of origin in FY 2007, and three bovine Tb cases lead back to the same herd in FY 2009. Each of these cases identified at slaughter were independently traced back to their respective herd of origin, thus the proportion of successful trace-backs (the unit of interest in our study) was not affected by these scenarios. It is important to keep this distinction

in mind because the epidemiological unit of interest at the national level for the bovine TB eradication/control program in the U.S. is the herd. Thus, from all the bovine TB cases successfully traced back to a herd of origin in the U.S. during 2001-2010 the majority (87%, 34 cases) were successfully traced back to unique herds, and only a small percentage (13%, or 5 cases) of bovine TB cases traced to a common herd. This is an important finding with implications for the evaluation of the national bovine TB eradication program in the U.S. This results indicates that the origin (herds) of the bovine TB cases identified in the U.S. in the last 10 years are herds without a previous history of bovine TB (they are new herds that were not detected before) rather than herds having a chronic bovine TB problem (residual infection) disclosing bovine TB cases at slaughter in different years. With regards our objective 3 (finding affected herds after a herd of origin was found as part of epidemiological investigations using the tuberculin test), our estimates would be slightly affected by these farms with 2 and 3 bovine TB cases detected at slaughter the same year. If we assume that these multiple bovine TB cases found at slaughter from the same herd of origin are part of a unique bovine TB outbreak in a herd in a giving year, and hence we exclude them from the calculations to obtain the risk of finding additional affected herds, the original overall 69% in our study reported would slightly decrease to 67%.

As previously mentioned, a limitation of the current epidemiological investigations (data) is that the number of "affected" herds found is not quantified in all epidemiological investigations, thus only a qualitative measure was recorded to indicate whether or not affected herds were found. I consider it important from an epidemiological point of view, as well as from the control aspect of bovine TB in the U.S., to record and make available relevant information related to the number of affected herds found (from each bovine TB case found at slaughter), the geographical location of each affected herd, the herd type, herd size and other important management factors, such as history of animal purchasing and selling. The availability of these information will facilitate future epidemiological analysis of bovine TB trace-back success and will also provide information for a more detailed and comprehensive epidemiological analysis including other factors relevant to the overall control of bovine TB in the U.S.

After conducting this study, which included an extensive review of the literature available on the U.S. bovine TB control and eradication program (objective 1), plus an extensive and detailed review of the epidemiological case files for the bovine TB cases found at slaughter during 2001-2010 (objective 2, 3 and 4), attending the bovine tuberculosis sessions of the 115th Annual United States Animal Health Association meeting (held Sept 29-Oct 5 in Buffalo, NY), attending the Conference of Research Workers in Animal Diseases (CRWAD) in Chicago during two years (2009 and 2010) where I presented and discussed my results, and finally interacting with USDA veterinarians and scientists interested in bovine TB in the U.S., it is my opinion that the same perceptions described more than 50 years ago by Anderson (1959a) as being widely accepted, may continue to serve as socio-economic impediments for the bovine TB eradication efforts today. These perceptions are as follows:

- an irreducible minimum has been reached with bovine TB and the U.S. beef and dairy industries will have to live with a certain amount of bovine TB;

- control measures are adequate;

- bovine TB is a vanishing disease and no longer an economic problem;

- bovine TB as a public health hazard is non-existent; and

- the bovine TB victory had been won, the need for vigilance is gone, pressures for vigorous action should be withdrawn and to continue pushing the eradication effort is a waste of time and money.

Due to the chronic nature of bovine TB, I would like to call for caution against belief in these perceptions. Our study results indicate that control measures are not adequate with respect to tracing bovine TB cases identified at slaughter since the majority of cases (66% or 78 of 117) with a herd of origin potentially in the U.S. were not successfully traced back (to the herd of origin), thus not enabling the disease, if present within the herd, to be controlled. Additionally, bovine TB has been documented as re-emerging disease worldwide, particularly in countries and regions that have infected wildlife populations. Some authors have cited that in countries and regions where wildlife populations live in close contact with cattle, the likelihood of successful eradication is compromised and possibly not feasible unless reservoirs are removed or the transmission of infection is resolved by means of a vaccine or by indefinite application of some other control measure (O'Reilly & Daborn, 1995; Collins, 1999). As previously mentioned, the U.S., in the states

of Michigan and Minnesota, has the added challenge of eliminating the last traces of bovine TB in the presence of a wildlife reservoir, white-tailed deer *(or Odocoileus virginianus*) (Schmitt *et al*., 1997; Kaneene, *et al.*, 2002). With an average 39 bovine TB cases being found per year (27 imported and 12 non-imported on average) (shown in Appendix D, Figure 10), it is dangerous to conclude that the battle against bovine TB has been won. If the relatively small amount of bovine TB that is presumed to currently exist in the U.S. is not controlled effectively, it has the potential to remerge and become a considerable economic problem. With the amount of bovine TB that is detected annually and the fact that unpasteurized (raw) milk sales is legal in 26 States (Singer, 2012), bovine TB as a public hazard still exists and illness related to the consumption of unpasteurized dairy products remains a public health threat in the U.S. today, as also indicated by Langer *et al*., 2012.

In conclusion, our results indicate that the State-Federal bovine TB control program can improve the "rate" of trace-back investigations to the herd of origin. Traces were successful for cattle with and without animal identification; however, the investigation process as it exists today, particularly when cattle lack official ID, is undeniably labor and time intensive. Each investigation requires the analysis of receipts and records, if available, from multiple premises. In scenarios where the bovine TB lesioned animal's owner cannot be determined, multiple producers must be tested with the CFT test at the government's expense. The producer whose herd tests positive for bovine TB is then presumed to be the owner of the bovine TB lesioned animal. Having to test multiple herds is inefficient and costly; with a better infrastructure for tracing animals in the U.S. this issue could be avoided and only one producer's herd would need to be tested, reducing the cost and increasing the efficiency of the epidemiological investigations.

Given that our results indicated that once a bovine TB lesioned animal was found at slaughter and a successful trace-back to the herd of origin was achieved the majority of the time further bovine TB infected animals (herds) were identified, it is crucial that State and Federal animal health officials are able to efficiently and rapidly trace animals (fed and adult cattle) back to their herd of origin (to all of their respective premises) when a bovine TB case is identified at slaughter. To do so, record keeping throughout the production chain (from birth to slaughter) needs to be improved and a simple, uniform method of trace-

back needs to be implemented (Wagner, 1988). Due to the variability in record keeping amongst the slaughter plants, feedlots, livestock markets, and farmers/producers, implementation of a uniform method of trace-back (i.e. a cohesive, fully implemented State and/or Federal official animal identification program) in the U.S. for cattle would greatly enhance the trace-back capability. The implementation of an official animal identification system would need to be able to trace cattle of all ages and types, from birth to slaughter, and would need to be able to trace animals within State lines and across State lines.

Achieving a higher "rate" of successful trace-backs to the herd of origin will ensure USDA officials have the background (starting point) to conduct epidemiological investigations needed to identify other potential exposed herds (animals) and remove additional infected animals. Implementing approaches and strategies to increase the "rate" of trace-back success should be a priority of the U.S. program. A higher rate of trace-back success would not only be beneficial to control bovine TB but could also be applied to the control and management of other diseases affecting livestock in the U.S.

This study shows that state and federal animal health officials are able to trace some bovine TB cases identified at slaughter back to their herd of origin under the current conditions; however, sufficient gaps exist that impair the capability for animal health official to trace *all* infected cattle. Standardizing the process of tracing cattle in the U.S. and enhancing the collection, storage and analysis of slaughter surveillance and epidemiological data would improve the efficiency and comprehensiveness of the program. The USDA should consider annually reporting how many granuloma submissions were confirmed bovine TB and how many confirmed bovine TB lesions were successfully traced back to a herd of origin. Currently the USDA annually reports how many affected herds were found in the U.S.; however, they do not provide context as to how many affected herds they could have found through slaughter surveillance but did not due to un-successful trace-back investigations (of confirmed bovine TB lesions).

In the U.S., slaughter surveillance provides an estimated animal-level prevalence of bovine TB that is highly dependent on the sensitivity of the method. The herd-level prevalence of bovine TB (number of herds in which infection is found) in the U.S. is dependent on detecting lesions at slaughter and

successfully tracing them back because systematic area testing is no longer practiced to the extent that it once was (USDA/APHIS/VS, 2009c). Thus, based on the results of this study and inherent limitations of slaughter surveillance, to draw conclusions regarding the U.S. national animal and herd prevalence solely based in slaughter surveillance findings (bovine TB cases detected), is not recommendable. In order to have an accurate estimate of animal-level and herd-level prevalence of bovine TB in the U.S. and in order for slaughter surveillance, the primary method of bovine TB disease detection in the U.S. to be an effective tool to control and eradicate the disease, it is of most importance that:

    1) all slaughter plants must meet the prescribed granuloma submission rate so that bovine TB lesions may be detected and submitted to the laboratory for analysis, and

    2) all bovine TB lesions detected at slaughter need to be successfully traced back to their herd of origin in order to maximize the detection of TB infected herds (identify sources of disease).

The success of the U.S. State-Federal cooperative bovine TB control and eradication program is dependent on the efficiency and comprehensiveness of these two steps. Failure to meet these steps will delay the successful control and eradication of bovine TB in the U.S. (Gilsdorf *et al.*, 2006).

In conclusion, the USDA has an opportunity to address the gaps identified in this study in order to increase the proportion of bovine TB cases that can be successfully traced back to a herd of origin. This will enable the bovine TB control program in the U.S. to be more successful in identifying infected herds (animals) thus, allowing the implementation of measures to control bovine TB in the U.S. Being able to achieve a higher level of traceability for cattle (and other livestock) will ensure the U.S. is well prepared to respond to any disease (domestic and/or foreign) that may threaten the livestock industry and public health in the U.S.

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Appendix

Appendix A: Example of distribution of bovine TB epidemiological investigations

"In January 2008, animal health officials from USDA and the California Department of Food and Agriculture (CDFA) expanded the epidemiological investigation of a large central California dairy herd that was infected with bovine TB. The disease confirmation was made in December 2007 following whole-herd tuberculin skin testing. The herd, composed of 5,016 dairy cattle, was depopulated. The ensuing investigation of this index herd resulted in the identification of 3,209 potentially exposed cattle that had moved to 143 other premises or to slaughter before officials knew that the herd was infected. Additional investigations to determine the origin of this herd's infection identified 110 cattle from 56 premises as potential sources for the disease. Epidemiological investigations conducted on the index herd during 2008 identified two other large dairy herds in California as TB-infected. One of these herds, which contained 1,014 dairy cattle, was depopulated. The other herd, composed of more than 12,000 cattle, is undergoing a test-and-removal program to rid the herd of TB. The resulting investigations of these 2 herds identified at least 14,410 potentially exposed cattle that, between 2003 and 2008, had moved to 354 other premises or to slaughter (whereupon they were subject to inspection by USDA's Food Safety and Inspection Service to ensure food safety). These movements required investigatory activities in 16 U.S. States and Canada" (USDA/APHIS, 2008b).

Appendix B: Cattle/meat marketing system in the U.S.
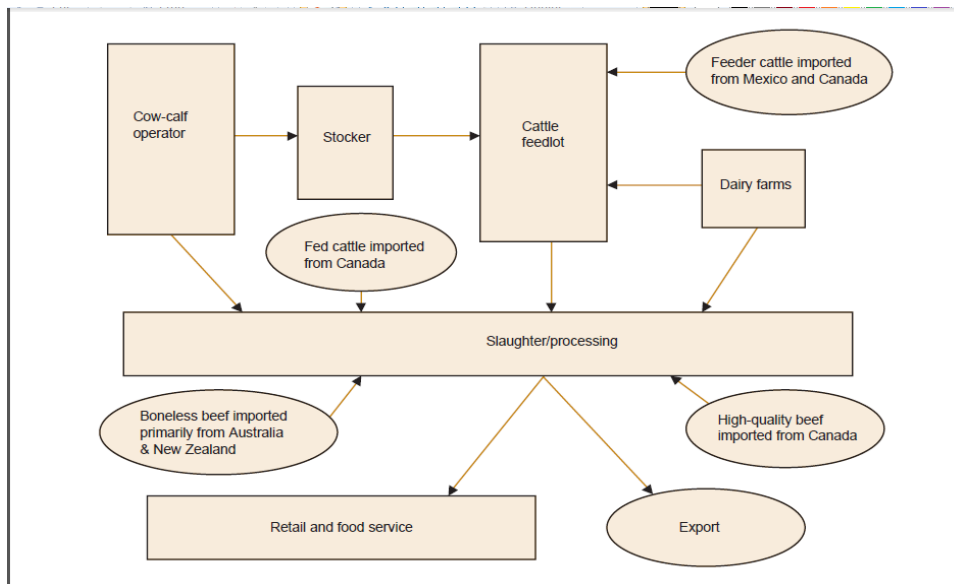

Figure 8: Cattle/meat marketing system in the U.S.

(Source: Golan *et al*., 2004)
(Note: There can be numerous permutations and combinations of this diagram)

Appendix C: Feedlot assessment of fed cattle disclosing a bovine TB lesion at slaughter

The graph in Figure 9 shows the number of bovine TB lesioned fed cattle per feedlot and shows that certain feedlots had more bovine TB fed cattle than others during the 2001-2010 time period. More specifically, for 2001-2010, seventeen feedlots had greater than 5 bovine TB lesioned fed cattle. The graph also shows that animal health officials should have been able to trace the majority of bovine TB lesioned fed cattle determined to be from Mexico back to these particular feedlots.

The graph in Figure 9 was created with the following logic and by the following steps. The owner names that were feedlots, the determination made for the country of origin, age of the bovine TB lesioned animal and a count of the bovine TB cases were the primary categories used for the pivot chart. It is important to know that the names in the owner column on slaughter surveillance spreadsheet originally came from the VS Form 6-35 filled out at slaughter. Since fed cattle are typically shipped directly from feedlot to slaughter, the owner name (owner of late) on the VS Form 6-35 and in the slaughter spreadsheet was typically a name with the word feedlot in it, indicating the most recent owner of the fed bovine TB lesioned animal was a feedlot. Fed bovine TB lesioned cattle with a name, but without the word feedlot in it, were researched to determine if the location was a feedlot. Any names found to be feedlots were included and non-feedlots were excluded. Since the owner/feedlot names on the slaughter surveillance spreadsheet are confidential, a new owner (feedlot) name index sheet was created in the slaughter spreadsheet where a unique number was assigned to each owner name that was a feedlot. Often a feedlot name would be listed several times on the slaughter spreadsheet main worksheet; however, it would be spelled slightly different each time. The unique number assigned to each feedlot name resolved this issue. In the future in order perform analysis using the owner names, the owner names will need to be spelled the in the same manner each time or a unique number needs to be utilized. The v-lookup command was then used to populate the main worksheet with the feedlot specific numbers. A pivot chart was then created in a separate sheet pivoting the following: ($\sum$ values: Count of Origin, Column Labels: Origin, Age, and Case considered bovine TB, Row Labels: Owner/Feedlot Number). The "Owner/Feedlot Number" was then filtered to exclude numbers that applied to locations that were not feedlots (private owners, livestock markets, etc.)

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and "Case considered bovine TB" was filtered by yes; consequently, the pivot only included only fed cattle. The pivot chart graphed 257 Mexican, and 60 non-imported (cattle with a herd of origin potentially in the U.S.), fed bovine TB lesioned animals that had a feedlot names listed as their owners on VS Form 6-35 and in the slaughter surveillance spreadsheet. Please note that the graph does not include all fed cattle that disclosed a bovine TB lesion at slaughter because not all were sold to slaughter from a feedlot. Some were sold to slaughter by a livestock market, private owner or broker/dealers.

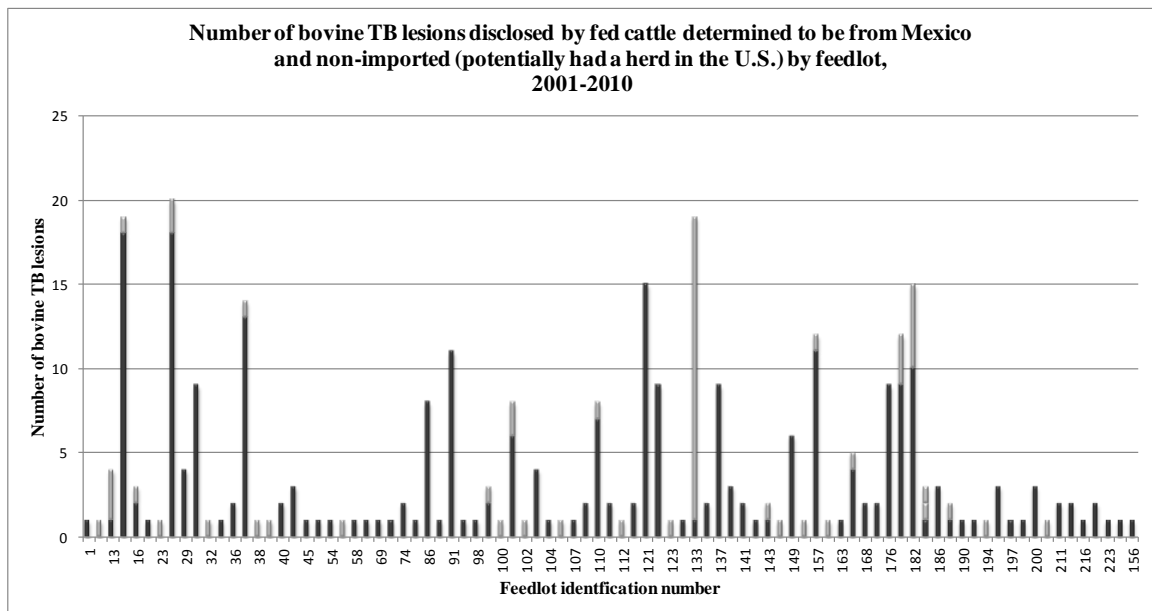Figure 9: Number of bovine TB lesions disclosed by fed cattle by determined to be from Mexico[1] and non-imported[2] by feedlot, 2001-2010

[1]Black = Lesioned cattle determined to be from Mexico
[2]Gray = Lesioned cattle determined to be non-imported (potentially had a herd of origin in the U.S.)
Note: The numbers on the graph do not line up properly with the vertical lines because the graph has been reduced in size.

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Appendix D: Annual analysis of bovine TB lesioned cattle identified at slaughter

Summary of bovine TB lesions determined to be imported and non-imported (potentially U.S.) by year

The number of bovine cases (lesions disclosed) per year by country of origin for 2001-2010 is shown in Figure 10 below. The number of bovine TB lesions disclosed per year by cattle determined to be potentially from the U.S. do not appear to be trending. On average 39 bovine TB cases are found per year (386 cases/10 years), of which 27 are imported (267 cases/10 years) and 12 are non-imported (119 cases /10 years), potentially having a herd of origin in the U.S.
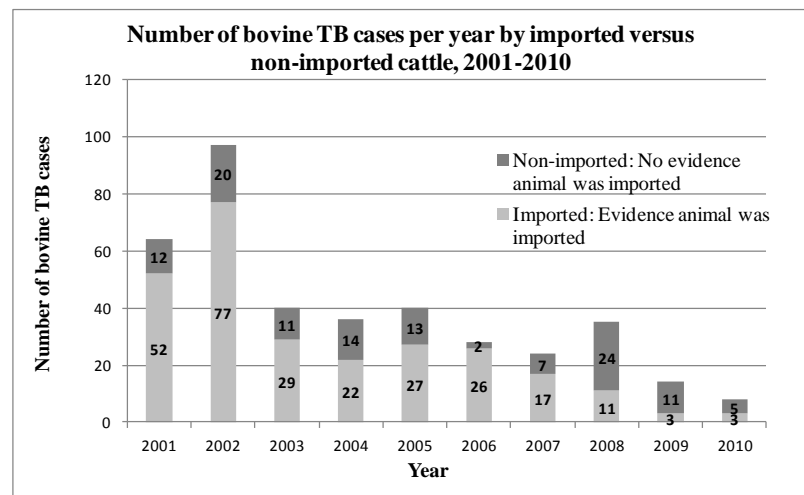
Figure 10: Number of bovine TB cases per year by imported versus non-imported cattle, 2001-2010

Note: The 21 fed cases in 2008 do not represent multiple exposures at different locations. These cases were from an outbreak where disease transmission occurred as a result of a point source exposure within a feedlot. Evidence of this exposure was confirmed through genotyping of the isolates (all of the isolates from these animals were all the same).

Bovine TB cases determined to be potentially from the U.S. by year as well as trace-back success

Figure 11 shows the number of bovine TB cases by year for cattle that potentially had a herd of origin in

the U.S. No trend can be seen related to the number of bovine TB cases per year.
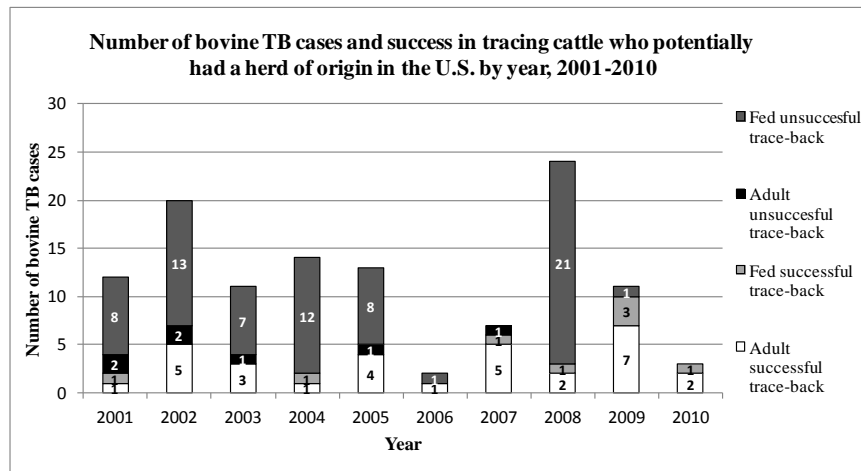

Figure 11: Number of bovine TB cases and success in tracing cattle who potentially had a herd of origin in the U.S. by year, 2001-2010

Note: The graph excludes 2 cases that were pending trace-back success
Note: The 21 fed cases in 2008 do not represent multiple exposures at different locations. These cases were from an outbreak where disease transmission occurred as a result of a point source exposure within a feedlot. Evidence of this exposure was confirmed through genotyping of the isolates (all of the isolates from these animals were all the same).

Bovine TB lesioned cattle that had no identification by year

Figure 12 below shows the number of bovine TB lesions disclosed by fed and adult cattle that had no

animal identification present at slaughter, by year for 2001-2010.   Despite having no identification, all of

the cases are investigation until a dead end it reached. Often animal health officials attempt to determine

the herd of origin by tuberculin testing all the herds belonging to the consigners that contributed to the

particular slaughter plant lot or pen where the lesioned animal resided prior to slaughter. If all of the

consignors herds test negative, then infection cannot be confirmed within a herd and the likely herd of

origin cannot be determined; consequently, the case is considered untraceable to a definitive location.
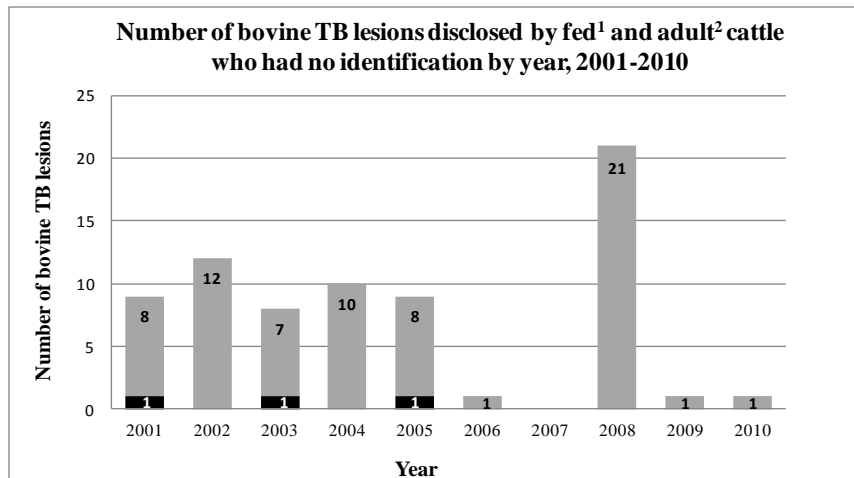
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Number of bovine TB lesions disclosed by fed[1] and adult[2] cattle who had no identification by year, 2001-2010

Figure 12: Number of bovine TB lesions disclosed by fed and adult cattle with no animal identification by year, 2001-2010

[1]Gray=Fed
[2]Black=Adult
Note: The 21 fed cases in 2008 do not represent multiple exposures at different locations. These cases were from an outbreak where disease transmission occurred as a result of a point source exposure within a feedlot. Evidence of this exposure was confirmed through genotyping of the isolates (all of the isolates from these animals were all the same).

Bovine TB cases determined to be from Mexico by year

Figure 13 shows the number of bovine TB cases (lesions disclosed) by fed cattle determined to be from

Mexico by year for 2001 to 2010. The number of bovine TB lesions disclosed by cattle determined to be

from Mexico has decreased over the ten year period.

Number of bovine TB cases from fed cattle determined to be from Mexico by year, 2001-2010
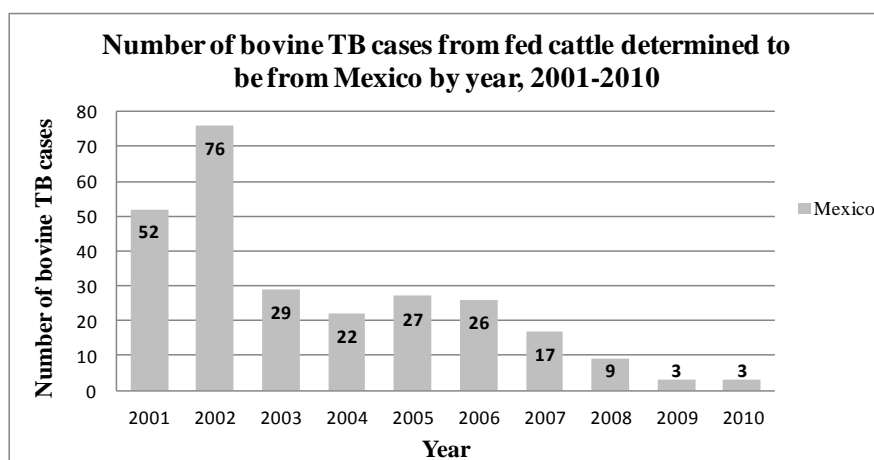
Figure 13: Number of bovine TB cases by fed cattle determined to be from Mexico by year, 2001-2010

Note: There were no adult bovine TB cases of Mexican origin

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Bovine TB cases determined to be from Canada by year

Figure 14 shows the number of bovine TB cases (lesions disclosed) determined to be from Canada by year for 2001 to 2010. The number of lesions disclosed by cattle from Canada was too low to detect a trend.
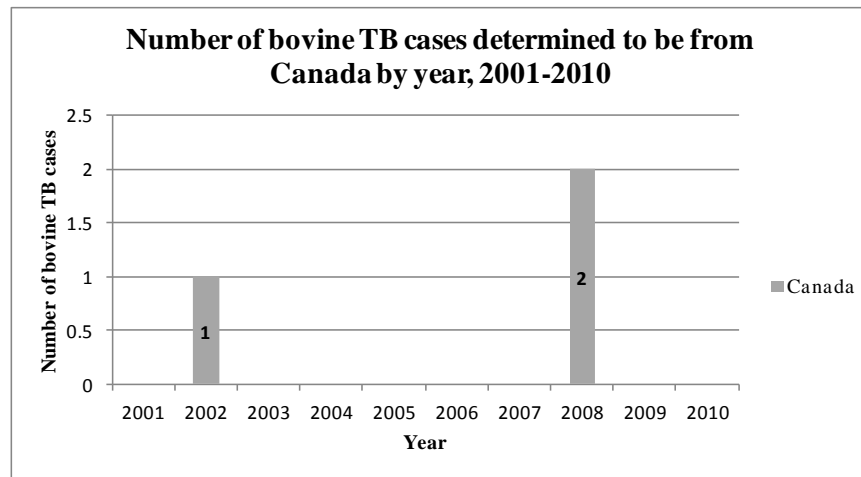

Figure 14: Number of bovine TB cases determined to be from Canada by year, 2001-2010

Appendix E: Presence of official and/or management ID or neither and association with trace-back success

Official and/or management ID or neither:

Table 19 show the frequency of having any identification (official ID and management ID, official ID only, management ID only) versus neither forms of ID for bovine TB lesioned cattle that had a herd of origin potentially in the U.S. and the proportion of these animals that yielded a successful trace-back to a herd of origin in the U.S. Seventy-six percent (or 89/117) of the bovine TB lesioned cattle detected at slaughter (that potentially had a herd of origin in the U.S.) had official animal identification and/or management animal identification. There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for bovine TB lesioned cattle with official ID and/or management ID (36%) compared to bovine TB lesioned cattle with no official animal ID and no management ID (29%); however, this difference was not statistically significant (p=0.28, OR=1.68 (95% CI 0.66, 4.29).

Table 19: Univariable analysis of trace-back success by official and/or management identification or neither for cattle with a herd of origin potentially in the U.S.

	Official and/or management ID or neither		
	Present	**Absent**	**Total**
Trace-back successful	32	7	39
Trace-back not successful	57	21	78
Total	**89**	**28**	**117***
Trace-back proportion	36%	29%	33%

*The trace-back success was pending for two of the 119 cases.

Official and/or management ID or neither by age:

Table 20 was performed to assess the association between official and/or management ID and trace-back success by age for bovine TB lesioned cattle with a herd of origin potentially in the U.S.

Fed bovine TB cases:

Of the fed bovine TB lesioned cattle detected at slaughter that potentially had a herd of origin in the U.S., 75% (or 59/79) had official ID and/or management ID present at the time of slaughter. There was the same proportion of successful trace-backs to a herd of origin in the U.S. for fed bovine TB lesioned cattle with

official ID and/or management ID (10%) compared to fed bovine TB lesioned cattle with no official ID and no management ID (10%); therefore, there was not a statistically significant difference (p=1.00, OR 1.02 (95% CI -4.38, 6.42)).

Adult bovine TB cases:

 Of the adult bovine TB lesioned cattle detected at slaughter that potentially had a herd of origin in the U.S., 79% (or 30/38) had official and/or management ID present at the time of slaughter. There was a higher proportion of successful trace-backs to a herd of origin in the U.S. for adult bovine TB lesioned cattle with official ID and/or management ID (87%) compared to adult bovine TB lesioned cattle with no official ID and no management ID (63%), however, this difference was not statistically significant (p=0.15, OR=3.9 (0.74, 21.4)).

Table 20: Presence of official and/or management identification or neither and the association with trace-back success by age for cattle with a herd of origin potentially in the U.S.

	FED			ADULT		
	Official and/or management ID or neither			Official and/or management ID or neither		
	Present	Absent	Total	Present	Absent	Total
Trace-back successful	6	2	8	26	5	31
Trace-back not successful	53	18	71	4	3	7
Total	**59**	**20**	**79***	**30**	**8**	**38***
Trace-back proportion	10%	10%	10%	87%	63%	82%

*The trace-back success was pending for two of the 119 cases.

Official ID and/or management ID or neither by gender:

To assess the association between official and/or management ID and trace-back success by gender for bovine TB lesioned cattle with a herd of origin potentially in the U.S., Table 21 was performed.

Male bovine TB cases:

Of the male bovine TB lesioned cattle detected at slaughter that potentially had a herd of origin in the U.S., 74% (or 55/74) had official ID and/or management ID present at the time of slaughter.   There was a higher

proportion of successful trace-backs to a herd of origin in the U.S. for male bovine TB lesioned cattle with

official ID and/or management ID (13%) compared to male bovine TB lesioned cattle with no official ID

and no management ID (11%); therefore, there was not statistically significant difference (p=1.00, OR 1.24

(95% CI -4.05, 6.53).

Female bovine TB cases:

Of the female bovine TB lesioned cattle detected at slaughter that potentially had a herd of origin in the

U.S., 79% (or 33/42) had official ID and/or management ID present at the time of slaughter. There was a

higher proportion of successful trace-backs to a herd of origin in the U.S. for female bovine TB lesioned

cattle with official ID and/or management ID (73%) compared to female bovine TB lesioned cattle with no

official ID and no management ID (56%), however, this difference was not statistically significant (p=0.42,

OR 2.13 (95% CI 0.50, 9.24)).

Table 21: Presence of official and/or management identification or neither and the association with trace-back success by gender for cattle with a herd of origin potentially in the U.S.

| | **MALE** | | | **FEMALE** | | |
| | **Official and/or management ID or neither** | | | **Official and/or management ID or neither** | | |
	Present	**Absent**	**Total**	**Present**	**Absent**	**Total**
Trace-back successful	7	2	9	24	5	29
Trace-back not successful	48	17	65	9	4	13
Total	**55**	**19**	**74***	**33**	**9**	**42***
Trace-back proportion	13%	11%	12%	73%	56%	69%

*The trace-back success was pending for two cases and sex was unknown for one case; therefore, only 116 (74 male and 42 female) of the 119 animals with a herd of origin potentially in the U.S. were available for analysis.

Appendix F: Presence of animal identification on cattle disclosing lesions at slaughter determined to be imported

Fed cattle disclosing lesions determined to be from Mexico

An important part of the Mexican national bovine TB eradication program is that blue ear tags are to be applied to all Mexican origin cattle slaughtered in the U.S. The blue ear tags contain prefixes indicating the "Mexican State of Origin" and have unique animal identification numbers associated with each tag (USDA-APHIS/FSIS internal document by Reed, 1999). Table 22 shows that of the 264 fed cattle from Mexico that disclosed a lesion at slaughter, 160 had official Mexican identification (a blue eartag) present at slaughter indicating their Mexican origin (60 had official Mexican identification and management tag, and 100 had official Mexican identification only) and 104 were determined to be of Mexican origin during the epidemiological investigation (61 had a management tag but no offical Mexican identication present at slaughter and 43 had neither official Mexican identfication or a management tag). If a bovine TB lesioned fed animal is determined to have been in a slaughter pen with cattle owned by a consiginer that only deals with Mexican cattle, animal health officials can reasonable conclude that the bovine TB lesioned animal was Mexican (eventhough the animal may be without animal identication). This was the case for some of the 104 that were determined to be of Mexican origin during the epidemiological investigation.

Table 22: Presence of animal identification on fed cattle disclosing lesions at slaughter that were determined* to be Mexican

Official Mexican identification present at slaughter? (i.e. DUS527068)	Management identification present at slaughter? (i.e. Yellow 135 or 407)	Number of infected cattle	USDA/APHIS/VS Investigation status/comments
Fed			
Yes	Yes	60	'Mexican origin', 'MX origin', or Mexican state listed
Yes	No	100	'Mexican origin', 'MX origin', or Mexican state listed
No	Yes	61	'Mexican origin per epi investigation'
No	No	43	'Mexican origin per epi investigation'
			or
			'Epi investigation shows most likely of Mexican origin'
	Total	**264**	

*Based on official Mexican animal identification and/or the results of the epidemiological investigation

Fed and adult cattle disclosing lesions determined to be from Canada

Table 23: Presence of animal identification on cattle disclosing lesions at slaughter that were determined*
to be Canadian

Official Canadian identification present at slaughter?	Management identification present at slaughter? (i.e. Yellow 135 or 407)	Number of infected cattle	USDA/APHIS/VS Investigation status/comments
Fed			
Yes	Yes	2	'Canadian origin'
Adult			
No	Yes	1	'Cow from Manitoba'
	Total Fed and Adult	3	

*Based on official Canadian animal identification and/or the results of the epidemiological investigation

Appendix G: Measuring the length of time for epidemiological trace-back investigations

The method used by VS to calculate the length of the epidemiological trace-back investigations involved counting the days from slaughter to case closure ("case closure date" minus the "slaughter date"). For the purposes of this study, "Slaughter dates" were available for all cases with a herd of origin potentially in the U.S.; however, "case closure dates" were only available for 14 cases. Furthermore, the "case closure date" was determined to be an unreliable date to use to calculate length of the investigations due to the lack of criteria (definition) with respect to what a "closed case" entailed. The use of the case closure date to mark the end of the investigation was also determined to inflate the perceived length of the investigation because it included the time required to complete administrative paper work (case notes). Due to these constraints, more accurate investigation start and end dates were determined to be the time between the slaughter date (since it was readily available for all cases) until either the date of the first tuberculin test performed on a suspect herd or date the cattle in affected herds were found PCR positive, where data were available. The number of days in between these dates were counted for the length of time in days to conduct the epidemiological trace-back investigation.

For future analysis, with respect to the investigation start date, since the start of the investigation is not until compatibility for Mycobacteriosis has been determined, using the date the lesion was received at the NVSL lab (1-2 days after slaughter date) or the completed might be more accurate than the slaughter date. If it is determined that the date the lesion was received for histocompatibility is a good start date for the investigations, VS would need to start populating the spreadsheet with these dates. Additionally, it may be advantageous for other purposes to track the date of the PCR results for the bovine TB lesion identified at slaughter (typically available within 3 to 7 days after the histopathology results) and the date of the culture results (4-8 weeks after the histopathology results).

For future analysis, with respect to the investigation end date, capturing the length of time it takes to trace-back and find the herd of origin (non-affected or affect herd) may be more informative than the length of time to case closure (when administrative paper work is completed); however, both lengths could be

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informative. The end of the investigation could be defined as the date of tuberculin testing of the identified herd (however, the herd of origin is sometimes not certain until infection is confirmed in the herd via the results from tuberculin testing, PCR and/or culture); therefore, the date of the results of tuberculin testing plus the PCR results and/or culture results may be necessary depending on the accuracy desired.

Lastly, consider combining the affected herd spreadsheet and the slaughter surveillance spreadsheet or link the two by the NVSL accession number to facilitate analysis using the date PCR positive, date culture positive, depopulation date and/or the quarantine release date. The length of time in days from date positive (tuberculin, PCR or culture) to the date depopulated maybe valuable to calculate and track. Additionally, the length of time in days from depopulated to quarantine release may also be valuable to calculate and track to ensure the recommended quarantine length is adhered to.

Appendix H: Select factors that could not be assessed

Animal factors (of the bovine TB cases identified at slaughter) that could not be assessed:

Animal type: An analysis could not be performed for animal type (cow, bull, heifer, steer) because the type was not always provided on the VS 6-35 form. Since it was not specifically requested on the VS 6-35, it was not consistently available in the slaughter surveillance spreadsheet. Animal type may be valuable factor to collect information on order to be able to analyze its association with the trace-back success and finding more bovine TB infected animals (affected herds).

Home-bred versus Purchased and Residency Period: Whether an animal is home-bred versus purchased and the length of it residency in a herd can be determined through contact with the producers during the epidemiological investigation; however, this information is not clearly stated and only occasionally mentioned in the epidemiological investigation files; consequently, it was not readily available in the spreadsheet for analysis purposes.

Breed: An analysis could not be performed for breed because it was not always provided in the "breed/color" box on the VS 6-35 form, the request for breed on the form allows color to be entered instead of breed and "breed/color" was not available in the slaughter surveillance spreadsheet.

Exact animal age: The analysis was not performed using the exact age of the bovine TB lesioned cattle that had a herd of origin potentially in the U.S. because the exact age was not available for all of them. Additionally, the preferred manner of entering the data as well as the manner in which the USDA wants to analyze it, are adult versus fed (greater or less than 2 years of age). If exact age is deemed important for analysis purposes, consideration should be given to specifying on the VS Form 6-35 whether age should be noted in number or simply fed versus adult.

Herd management factors (of the bovine TB cases identified at slaughter) that could not be assessed:

Type of herd: The herd type (dairy, beef) that the bovine TB lesioned animal(s) came from was not requested at the time of slaughter inspection and therefore was not noted on the VS 6-35 (and may or may

not have been known at the time of slaughter). If the epidemiological trace-back investigation was

successful, the type of herd the animal came from was noted on the tuberculin test record. Despite some

cases being unsuccessfully traced, the herd type was sometimes determined or presumed based on the

investigation process. However, overall, the type of herd was not available on the VS Form 6-35 for all

cases (with a herd of origin potentially in the U.S.) nor was it readily available in the slaughter surveillance

spreadsheet for analysis purposes. For this study, an attempt was made to fill in the herd type for as many

cases as possible using the affected herd spreadsheet and the case files; however, there was not enough

information to make this determination for all cases (with a herd of origin potentially in the U.S).

Consequently, I was not able to analyze this factor.

Herd size: In the U.S., the size of the herd(s) of the bovine TB lesioned animal was not known at the time

of slaughter and could only be determined if the epidemiological trace-back investigation was successful.

For some cases, the herd size was indicated on the 'affected herd spreadsheet'; however, it was not

available for all cases and was only an estimate for many of them.

Time interval between previous herd test and factory initiated herd test of the herd of origin: Currently in

the U.S., the time interval between the previous test of the herd of origin herd (the last accredited herd test)

and the factory initiated herd-test of the herd of origin (after the bovine TB lesion was found at slaughter),

can only be determined once the herd is successfully located as a result of the epidemiological

investigation. Even then, there is a possibility that the herds may have never had a previous herd test.

Appendix I: Data elements in the slaughter surveillance spreadsheet: how they were modified for this study and how they could be modified to facilitate future analysis

1) **State Primary** and **State Secondary** – To avoid occasional mix ups with "State Primary" and "State Secondary", I recommend adding five columns: "**Establishment State**", "**Shipper State**", "**Owner State**" and "**Market/Buyer State**" to match the 6-35 and a "**State traced to**" column. The "State traced to" should be populated with the State the investigation traced, since the Owner State is not always the herd of origin state. If the investigation leads to multiple states, add another "State traced to" column, do not put two states in one column because that will prohibit analysis. Any cells that are not applicable should be left blank, put a missing value (.) or n/a. The additional five columns will provide support for what is in the primary and secondary columns and will facilitate analysis. Consider deleting state primary and secondary in the future.

2) **Official animal ID** - Add this column to track whether the animal had official ID. Use a "0" (or "No") if there was no official animal ID and a "1" (or "Yes")if the animal had a brucellosis vaccination tag, Backtag, Bright tag, NAIS/RFID, Mexican ID, and/or Canadian ID. Make this determination based on what is entered in the existing "ID" column.

3) **Management ID** - Add this column to track whether the animals had management ID (generic bangle tag used to manage herd). Use a "0" (or "No") if there was no management ID and a "1" (or "Yes") if the animal had management ID. Make this determination based on what you enter in the existing "ID" column.

4) **Country or State of Origin** - This column already exists but I would split it into two columns: one for "**Country of origin**" and one for the "**Mexican state of origin**". In its current state the column is tracking two (overlapping) things in one column. After breaking them out, you could delete the "Country or State of Origin" column since it will be redundant.

For this project to sort and pivot with only country of origin (not MX state), a column called "**Origin**" was added with "0" for unknown origin, "1" U.S. origin, "2" Mexican origin, and "3" Canadian origin. This newly created column could be deleted, since it will be redundant to the "Country of origin" column; however, "Origin" was used in many of the pivot tables and charts. For use of these pivots table and charts in the future keep "Origin" or re-create the pivots using the new "Country of origin".

5) **Owner** – Variations in the spelling of the owner names made it difficult to analyze this column for the feedlot pivot chart/graph. In the future, spell the owner & feedlot names as consistent as possible for analysis purposes. Reference the "feedlot name&num" sheet (newly created for this project) for various spellings of the owner/feedlot names and choose a format/spelling to adhere too. Another way to deal with this issue is to populate an owner/feedlot "number" column.

6) **Owner/Feedlot number** – Add this column to create pivot charts of cattle by feedlot.

7) **Animal type** - Added a column called Animal Type to indicate whether the case was 'bovine' or 'cervid'. This column was added for this project to count/sort/pivot by (only) bovines.

8) **Age** - This column already exists but split it into two: one for "Age" and one for whether the case was considered to be bovine tuberculosis. Instead of using the "+" signs to indicate whether the animal was *M. bovis* have a separate column for this. Restricting the column(s) to one purpose keeps it cleaner when analyzing the data. Instead of the "+" signs, put a "yes" or "no" in a column labeled "**Case considered BTB**" based on the histopathology, PCR and culture results. A 'yes' in this columns would signify that the case warranted an epidemiological investigation. For this project, this column was added because some cases did not have a PCR value (blank or not requested) and/or did not have a culture (blank or NIM) yet they were investigated. In many of the pivot tables, this newly created column was used to count "Cases considered BTB" without the age determination. This separation also enables age to be analyzed without knowing whether they were fed+ (and fed), and adult+ (and adult), etc with unk+ and cervid+.

9) **Sex** - This column already existed but consider splitting it into two columns. This cell was traditionally populated with whatever was in the "sex" box on the Form 6-35 (sometimes female and other times heifer or cow); however, this creates some confusion when trying to analyze the data. Restrict the "**Sex**" column to "male", "female", and "unknown" and create a "**Bovine type or class**" with the values: bull, cow, heifer, steer, unknown. Also, choose either "Fe" or "Female" and stick with that determination for consistent results when sorting and pivoting.

10) **Trace-back** - Add this column to track when epidemiological investigations successfully trace back to a herd of origin. Using "0" and "1" (or "no" and "yes") make it easy to count and analyze the data. Use "0" for unsuccessful, "1" for successful, "." for cases that were not bovine TB, and "TBD" for cases still under investigation.

11) **Affected herd found** - Add this column to track epidemiological investigations that trace back to a herd of origin and subsequently perform testing and find infection within the herd. Use a "0" (or "no") when an affected herd was <u>not</u> found and a "1" (or "yes') when at least one affected herd was found.

12) **Type of herd** – Add this column to track and analyze the type of herd the bovine TB lesioned animal came from. Use "beef", "dairy", and "unknown". Consider deleting the newly added "**Roping**" column and have "roping" as an option under this column or it may be beneficial to have both the Type of herd and the Roping columns.

13) **The Affected Herd Spreadsheet** – Add a "**NVSL accession number**" column for the affected herd(s) found via slaughter surveillance so the two spreadsheets can be linked by a common value or add 'herd type', 'herd size', 'date PCR positive', and 'date culture positive' to the slaughter surveillance spreadsheet to facilitate analysis.

Appendix J: Recommendations related to the collection, storage and analysis of slaughter surveillance data

Our analysis re-emphasized shortcomings in data collection and storage systems related to bovine TB cases. An integrated data system would greatly improve the ability to thoroughly and accurately address questions about the bovine TB surveillance system in a timely manner, as Meyer stated in 1988. Our recommendations for the USDA related to the collection, storage and analysis of slaughter surveillance data are as follows:

1) The slaughter surveillance spreadsheet, that national TB staff maintains, indicated (by red highlighting) the bovine TB cases that were successfully traced to a herd and an affected herd was found; however, encoding this data would allow for more efficient and clear:

a) quantification of how many cases were successfully traced back versus not successfully traced back, and

b) determination of cases were successfully traced back but an affected herd was not found.

For this study we had specific criteria for what was a successful and an un-successful trace-back to a herd of origin. As such, we recommend the USDA establish specific criteria for what they consider to be a successful versus an un-successful trace-back to a herd of origin and that they annually quantify and report the number of successful and un-successful epidemiological trace-back investigations. Additionally, consider assessing trace-back success for trends over time and by region or State.

2) Instruct animal health officials to specifically document whether they found the birth herd versus another herd (the most recent herd, interim herd, or herd of primary residence) so that it may be tracked in the slaughter surveillance spreadsheet. If it is not possible to determine what herd was found (birth herd, herd of primary residence, etc.), then that should be recorded. The type of herd (beef, dairy, mixed, rodeo) should also be clearly recorded.

3) Code data where applicable to facilitate analysis. For example, based on the results of the histopathology, PCR and/or culture, code whether the case (overall) was determined to be bovine TB or not. In instances were a case is histopathology = Compatible for Mycobacteriosis, PCR = *M. avium* and culture = *M. avium*, codification of the data would allow the case to easily be excluded from analysis.

4) Periodically assess whether animal identification available at slaughter is related to trace-back success. Continue to manually enter all animal identification numbers into slaughter surveillance spreadsheet from the VS Form 6-35 to facilitate analysis using animal ID. Consider having separate columns for official animal identification versus management identification.

5) For each successful trace-backs, enter (in the slaughter surveillance spreadsheet) the State(s) where the investigation(s) took place and the States where the investigation terminated (in supplement or replacement of the state primary, state secondary). This will help facilitate analysis of how many bovine TB lesions were disclosed in each region and State.

6) Retain epidemiological investigation case files (paper and electronic) for 7 years. Out of the total case files examined to complete our objectives, 23 paper case files from 2001-2005 could not be found.

7) Only categorize bovine TB lesioned animals as Mexican when official Mexican identification was present at slaughter (See Appendix F, Table 22 for more information). Consider adding a "highly suspected as being of Mexican origin" category in order to be able to differentiate between cattle that were conclusively Mexican and those that were not.

8) For current and future analysis purposes, ensure the slaughter surveillance spreadsheet is tracking all pertinent information from the VS Form 6-35, laboratory reports and epidemiological investigation case files.

- Review nomenclature in the slaughter surveillance spreadsheet and modify it to match the VS 6-35 or laboratory reports. For example, to facilitate uniformity, instead of state primary, state secondary, animal ID, etc. use "establishment state", "market/buyer state", "owner state", "ear tag/other official permanent ID#", "sale/back tag #", and "other ID" (as mentioned above).

- Review the information collected during the epidemiological investigation process and determine if additional data should be tracked in the slaughter surveillance spreadsheet in order assess the efficiency and effectiveness of the bovine TB control program over time. Request that AVIC's/VMO's/Area Epidemiologists specifically/clearly document information determined to be pertinent for analysis purposes in the cases files so it may be transferred into the slaughter surveillance spreadsheet easily.

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9) To facilitate the uniform collection of data, consider changing the VS Form 6-35 to contain more check marked / boxed options versus the existing blank boxes that need to be filled in. For items that require additional information, leave space for notes.

- For example, for sex provide male/female as the only options, for breed provide the options for the most common types and leave a blank to be filled in for other, for age decide if numbers or fed/adult should be used (if fed/adult only provide these two options only).

- Consider adding boxes for bovine type (heifer, steer, cow, bull, rodeo) and beef/dairy if it is possible that this information could be known at slaughter.

- Make sure the space for animal identification is wide enough for the data.

10) Ensure that all archived epidemiological case related emails and attachments can be opened once archived (some email attachments were not viewable after being archived).

11) Periodically assess what feedlots bovine TB infected fed cattle come from. For feedlots that have substantial numbers of infected cattle, assess whether it is a regional issue. Determine if any additional precautionary measures should or could be taken for feedlots found to have substantial numbers of bovine TB lesioned cattle.

12) Conduct a requirements study to identify the benefits that could be derived from having a centralized, password protected, relational database with or without a hand held device (such as a personal digital assistant (PDA)) that could facilitate and validate the entry of slaughter surveillance and epidemiological case information. As a part of the requirements study, review the issues that were encountered when the Tuberculosis Information Management System (TIMS) database was used. Access what information would be most valuable to be collect and analyze from the slaughter surveillance process and the epidemiological investigations. A database and hand held application for entering slaughter surveillance and epidemiological information could minimize the time required to gather and query data for bovine TB and other diseases, as well as, improve the quality of the data entered. The portable hand held device would eliminate the double step(s) of having to write the slaughter and epidemiological information on a paper and then having to manually enter the data into spreadsheet at a later date. The PDA could even use 'Imagine processing' software to take image of the animal identification number(s) and convert them to

alpha numeric data, which could be stored in a database. It is understood that the low number of cattle detected at slaughter with bovine TB may not justify such an application; however, it could be justified if it was designed to include surveillance for other diseases in addition to bovine TB. Despite the low number of cases of bovine TB detected each year, analysis of the disease will continue to be hindered if the manner in which the data are currently collected and stored remains as is.

Appendix K: Case numbers for bovine TB cases for which further evaluation is recommended

Further evaluation of available information are recommended for the following four, 2005 cases: 390650,

383657, 383654, 383653 and one, 2004 case: 330945.