The Committee met on November 15-16, 2010 at the Hilton in Minneapolis, Minn., from 1:00 to 6:00 p.m. and 8:00 a.m. to 12:00 p.m., respectively. There were 52 members and 36 guests present. Introductions of Vice-Chairs and Subcommittee Chairs were made. An overview of the 2009 meeting and resolutions were given.

Presentations and Reports

Dr. Phil Elzer presented the Scientific Advisory Subcommittee Report, which is included at the end of this report.

Dr. Carter Black Feral Swine Subcommittee Report, which is included at the end of this report.

The Greater Yellowstone Area (GYA) Subcommittee Report was presented by Dr. Marty Zaluski, and is included at the end of this report.

FY10 US Cooperative Brucellosis Eradication Program Update
Dr. Arnold Gertonson, USDA-APHIS-VS

A summary of this presentation is included at the end of this report.

Future of the US Brucellosis Program
Dr. Mike Carter, USDA-APHIS-VS

In an effort to maintain forward momentum with the cooperative Federal-State-Industry effort to eradicate bovine brucellosis, Veterinary Services’ (VS) developed a concept paper that was made available for public comment in the Federal Register that describes our approach to addressing ongoing challenges to the brucellosis program. The concept paper provided a framework to: 1) effectively demonstrates the disease-free status of the United States through a national status-based program supported by a national surveillance strategy; 2) enhances efforts to mitigate disease transmission from wildlife; 3) enhances disease response and control measures; 4) modernizes the regulatory framework to allow VS to address risks quickly and sensibly; and 5) implements a risk-based disease management area concept.

To move forward, USDA-APHIS-VS has drafted an interim rule which has gone through clearance but a publication date has not been determined. The draft interim rule will remove the automatic loss of Class Free status in any Class Free State if a brucellosis-affected herd is not depopulated within 60 days or if two or more herds are found to have brucellosis within 24 months. The State will retain Class Free status if 1) affected herds
are maintained under quarantine, 2) an individual herd plan, including a test-and-remove schedule, is developed and implemented for each affected herd to prevent the spread of brucellosis, and 3) appropriate surveillance is conducted to detect brucellosis in other herds or species.

The draft interim rule will remove certain surveillance requirements for States or areas that have been Class Free for 5 or more years and do not have *Brucella abortus* in wildlife. The changes in surveillance requirements being removed include eliminating the twice-yearly ring testing of dairy cattle herds and the elimination for each State to collect blood samples from 95 percent of all cows and bulls 2 years of age or older. Instead, all recognized slaughtering establishments in such States or areas must agree to participate in slaughter surveillance testing as part of a new national bovine brucellosis surveillance plan VS is developing. These changes will eliminate redundancies in current slaughter surveillance testing and increase the efficiency of the bovine brucellosis slaughter surveillance program.

In order to mitigate the potential risk of transmission of brucellosis from brucellosis affected herds in Class Free States, the interim rule will require any Class Free State with *B. abortus* in wildlife or continued detections of brucellosis-affected herds to develop and implement a brucellosis management plan (BMP) approved by the Administrator. The BMP will: 1) Define and explain the basis for the geographic area identified in the BMP, 2) Describe surveillance activities for domestic cattle and bison and, if applicable, wildlife, 3) Describe mitigation activities for both domestic cattle and bison and wildlife within or from the BMP, and 4) Describe epidemiologic assessment and surveillance activities to determine if wildlife populations are affected. BMPs that do not address wildlife must describe epidemiologic activities that demonstrate wildlife populations are not a source of the disease.

As USDA-APHIS-VS develops new regulations for the brucellosis program, we will continue to engage a wide range of stakeholders and other interested parties for input on the proposed strategies, program standards, surveillance plans, and other policy concepts. In order to develop a regulatory framework to present to the public, USDA-APHIS-VS has formed a Joint Tuberculosis and Brucellosis Regulatory Working Group. Because the bovine tuberculosis program is undergoing similar changes, VS is proposing to create a single rule for both the bovine tuberculosis and brucellosis programs. The working group membership includes State Tribal and Federal animal health representatives. Developing the proposed regulation will take up to 2 years.

**Status of the Campaign Against Brucellosis in Mexico**
Dr. Jose Alfredo Gutierrez, CGRPA, Mexico

A summary of this presentation is included at the end of this report.

**Select Agents – Should *B. abortus* be Listed?**
Dr. Thomas Myers, USDA-APHIS-VS

**Biennial Review**
- The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires APHIS and CDC to conduct a biennial review of the list of select agents and toxins and to revise the list as necessary. The Agencies evaluate each agent using a method developed in accordance with the regulations. This process involves bringing together scientific government experts to evaluate each agent using certain criteria. The last review was completed and published in the *Federal Register* on October 16, 2008.
- Both APHIS and CDC have received recommendations from their respective scientific review committees and are considering those recommendations.
- We are also considering comments that we received on the advance notice of proposed rulemaking (ANPR) that we published in the *Federal Register* on July 29, 2010. We asked for public comment on changes to the list of select agents and toxins and tiering of the agents.

**Federal Advisory Panel**
- The panel will advise the joint APHIS and CDC Select Agent Program on security matters related to biological agents and toxins, including specific recommendations regarding the addition, retention, or deletion of listed select agents and toxins.
- The panel formed 3 working groups to leverage the varied expertise of the public health, animal health, scientific, security, and intelligence communities to assist in developing these recommendations. These working groups developed recommendations on:
  - The select agent list and tiering
  - Personnel reliability
  - Physical and cyber security
The panel recently completed its work and is sending their final recommendations to the Secretaries of USDA and HHS.

**Regulatory revisions**

- APHIS and CDC will consider the FESAP recommendations as well as the comments received to the ANPR.
- We realize that there has been considerable interest in removing *B. abortus* from the select agent list to facilitate research in large-animal vaccine studies.
- In evaluating this agent, we must consider carefully the threat that brucellosis agents pose to animal health. Furthermore, it is important to note that *B. abortus* is also on the CDC’s select agent list. To ease restrictions on working with the organism, it would have to be removed from both lists.
- We will continue to work with CDC to provide a timely and consistent review process in approving work with this agent in laboratories and animals.
- Under the Executive Order’s requirements, any proposed changes that are made to the list of select agents and toxins will be promulgated as a final rule in the regulations by October 2011.
- Therefore, after considering the FESAP recommendations and ANPR comments and other comments we have received to date, we anticipate publishing our proposed rule in early 2011. This proposed rule will allow for additional public comment.

**Herd Depopulation Matrix**

Dr. Mike Gilsdorf, National Association of Federal Veterinarians

Dr. Gilsdorf presented a proposed Brucellosis Infected Herd Depopulation Decision Matrix which had been suggested by the Greater Yellowstone Area (GYA) Subcommittee.

The rationale is to standardize the decision process used to determine whether or not to depopulate a Brucellosis infected herd with the goal of creating some flexibility in handling affected herds. The GYA Subcommittee’s concept employs a prioritized and point weighted list of factors with a depopulation decision based on objective point value and in consultation with the state animal health official.

- Most important – point value of 5
  - Available funds – federal and state
  - Risk to other herds
    - **Ability of quarantine to be maintained**
    - Proximity
  - Costs of testing and vaccination
  - Herd Plan Compliance

- Next most important – point value of 4
  - Herd size
  - Time since probable infection
  - Seroprevalence in herd
  - Commingling with infected wildlife
  - Presence of abortions and infertility
  - Source of infection

- Less important – point value of 3
  - Closed or open herd
  - Infection found before or after calving

- Next least important – point value of 2
  - Status of area
  - Infection found before or after going to grazing
  - Poor bio-security measures

- Least important – point value of 1
  - Ecology

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Seroprevalence in herd (3% or greater)
Appreciable presence of abortions and infertility
Commingling with infected wildlife
Source of infection: cattle
Open herd
Infection found after calving
Intent of area (DSA or below Class Free)
Infection found after going to grazing
Poor biosecurity measures
Ecology
TOTALS

Development of Standardized Risk-Based Process for the Evaluation of a Brucellosis Management Area
Dr. Katie Portacci, USDA-APHIS-VS
A summary of this presentation is included at the end of this report.

Montana Review
Dr. Marty Zaluski, Montana State Veterinarian
Montana is focusing its brucellosis efforts in four priority areas.
1) The state is continuing to test a large number of cattle and domestic bison with approximately 60,000 samples tested since November 2009. Sampling is highly cyclic with a large surge in October and November due to the shipment of weaned calves out of surveillance area.
2) The Designated Surveillance Area (DSA) has been in Montana regulations through an official order enacted by the Board of Livestock in January 2010. These regulations are being drafted into rule with the public comment period taking place between October 28 and November 30, 2010.
3) On November 1, Montana received notice from National Veterinary Services Laboratory of a culture confirmed positive brucellosis herd. This herd, affected with Brucella abortus biovar 1 is a large domestic bison herd South of Bozeman, and is located in the Montana’s DSA.
4) Montana Department of Fish Wildlife and Parks is commencing on a multiyear study to live capture 500 elk over five years in the boundary area of where brucellosis is known to exist. As part of the study, positive elk will be collared and implanted with vaginal transmitters. Abortion or birth sites will be located for culture. Elk remaining seropositive at the end of study period will be removed.

Idaho Review
Dr. Bill Barton, Idaho State Veterinarian
A positive brucellosis herd was discovered in eastern Idaho in late November 2009 as a result of slaughter surveillance. The herd, located in the Rigby area, consisted of 589 mixed breed beef cows. Three (3) cows from the herd were confirmed by the National Veterinary Services Laboratory to be positive for Brucella abortus biovar 1 on milk culture. The herd was immediately put under quarantine and a whole herd test completed. The remainder of the herd tested negative for brucellosis. Reactor animals identified in the initial whole herd test were sent to slaughter on December 15, 2009.

Due to the unique management factors associated with this herd, the state recommended whole herd depopulation and offered to assist with indemnity funding. Negotiations continued through January with USDA APHIS VS to secure indemnity funding to allow depopulation of the herd. USDA Under-Secretary Avalos issued a final decision on January 19th disallowing federal indemnity to depopulate the herd. As a result, the herd remained under quarantine, test and remove management.

During the month of March, 2010, three cows within the affected herd aborted calves. One cow and her aborted fetus were culture positive for field strain brucellosis. A whole herd test was completed and three additional reactor cows were identified. The four (4) newly identified reactors were sent by permit on a Form VS1-27 to the National Wildlife Research Center in Fort Collins, Colorado for research purposes.

A herd plan was drafted and submitted to USDA APHIS VS for comment and approval. The herd plan separated the main herd into two (2) smaller herds for management purposes. The smaller herd consisted of 161 head of younger breeding cows and calves. This breeding herd could potentially be allowed to go onto summer grazing should quarantineable pasture be located. The second herd, consisting of older cows with some calves at side, was to be held under quarantine in a dry lot/pasture combination until the calves were old enough to wean. Some open cows from this group were voluntarily sent to slaughter on Form VS1-27 during the late spring/ early summer months with no federal or state indemnity.
In early May, 2010 a conference call was held with the USDA APHIS Administrator, USDA APHIS VS Chief Veterinary Officer, ISDA Director, ISDA Deputy Director and the Idaho State Veterinarian to discuss the brucellosis affected herd, the challenges associated with maintaining a quarantined herd and the financial effect on the herd owner.

On May 28, 2010, the breeding herd of 161 pair was allowed to go to summer pasture on a USFS allotment under quarantine. The selected allotment had no fence line contact with other cattle herds and no other herds in close proximity.

On June 3, 2010, USDA APHIS VS reversed their position and agreed to allow depopulation of the breeding herd with federal and state indemnity. 156 head of cows and 4 bulls were sent to slaughter on June 23, 2010. Five (5) cows were not recovered from the grazing allotment until early August. Those five cows were slaughtered on August 13, 2010.

On September 9, 2010, the remaining adults in the second management herd were voluntarily sent to slaughter with no federal or state indemnity.

The 2009 bull calves were castrated and the heifer calves were spayed. These animals were released from quarantine.

The 2010 bull calves were castrated and the heifer calves will be held under quarantine until they have been spayed or sent direct to slaughter.

In late spring 2010, the herd owner purchased 58 fall calving cows from a single source. These cows were all test negative prior to purchase but became part of the affected herd. A pre calving brucellosis test of this herd was completed on August 24, 2010, and all animals were negative. A post calving brucellosis test was completed on October 28, 2010 and all cows tested were negative. This herd will remain under quarantine and the brucellosis testing regimen will continue until all appropriate testing has been completed and the herd can be released from quarantine.

The epidemiological investigation included brucellosis testing 1087 head of cattle from 6 source herds (all negative), 3086 head of cattle from 20 potentially exposed herds (all negative) and re-testing 1479 head of cattle from 8 potentially exposed herds (all negative).

Although a definitive source of infection for this herd has not been determined, four (4) *Brucella abortus* biovar 1 isolates recovered and genotyped from two (2) of the infected animals are most similar to strains recovered from a wild elk in Idaho, a wild elk in Montana and an affected cattle herd in Idaho in 2002.

Wyoming Review
Dr. Jim Logan, Wyoming State Veterinarian, Wyoming Livestock Board

Wyoming found a new case of *B. abortus* in a cattle herd in Park County, east of Yellowstone National Park (YNP), in late October 010. Wyoming has a good surveillance program that requires testing within thirty (30) days prior to change of ownership or movement on test eligible females originating within our Designated Surveillance Area (DSA). Three reactor cattle were identified on the required test at a Wyoming livestock auction market and results verified by the Wyoming State Veterinary Laboratory (WSVL) and the National Veterinary Services Laboratory (NVSL).

Within two (2) weeks of notification of these reactors, we have whole herd tested the herd of origin and found one additional reactor, quarantined all adjacent and contact herds and to date have tested 85% of these herds. Over 3000 head have been tested in relation to this case with no additional infection being found.

On November 9th, we were notified that *B. abortus* Biovar 1 was cultured at both WSVL and NVSL from tissues collected from one reactor. Wyoming is nearing completion of the epidemiologic interviews with quarantined herd owners and is in the process of developing quarantine release herd plans. The infected herd will remain under quarantine pending future whole herd testing. The most likely cause of this infection is wild elk in the Greater Yellowstone Area (GYA). Genetic comparison of the bacterial culture with previous case culture positives (elk and cattle) will be done to attempt to verify the source. This case is within Wyoming’s DSA and occurred approximately 50 miles east of YNP in an area over 100 miles away from any elk feedgrounds.

Wyoming expects to find sporadic cases of Brucellosis in our cattle herds as long as the wildlife reservoir exists in our state and we are committed to deal with these appropriately to prevent spread of the disease. Our test and identification requirements provide good surveillance, traceability, and early detection.

Wyoming Game and Fish Review
Dr. Jim Logan on behalf of Dr. Terry Kreeger, Wyoming Game and Fish Department

Test and Slaughter

In an effort to reduce prevalence of brucellosis among elk, the Wyoming Game and Fish Department implemented a pilot project using test and slaughter on three feedgrounds in the Pinedale elk herd unit from 2006 to 2010. Seroprevalence of antibodies to *B. abortus* of elk captured from the Muddy Creek feedground fell from 37% (*n* = 158) in 2006 to 5% (*n* = 141) in 2010 with the slaughter of 107 seropositive animals. Although at least
two trapping attempts were conducted every year at Muddy Creek feedground, cumulatively only 646 of 1,321 (49%) adult and yearling female elk available were captured and tested. Slaughter of seropositive elk at Muddy Creek did not appear to prevent brucellosis transmission events based on serology and culture data. Lesser brucellosis seroprevalence reductions were also observed on the Fall Creek and Scab Creek feedgrounds following removal of 32 and 58 seropositive elk, respectively.

**Surveillance**

2009 Brucellosis surveillance in non-feedground elk was focused on the Cody area where seroprevalence has increased over the last few years. In addition, continued statewide and selected feedground (see BFH below) surveillance was also conducted. Target areas included the Snowy Range in southeastern Wyoming, hunt area 102 in the southwestern corner of the state, and the Wind River Indian Reservation (HA 127). The target areas for 2009 completed statewide coverage, which began in 2005.

Serological analysis was initiated on blood samples received from this year’s brucellosis surveillance. Of the 6,000 blood collection kits sent to hunters successful in drawing limited quota elk licenses, 822 samples were returned to the laboratory, with 483 being suitable for testing.

**Brucellosis-Feedground-Habitat**

**Surveillance**

A total of 662 elk were trapped and 401 newly tagged at 11 feedgrounds during the 2009-2010 winter. A total of 406 test-eligible female elk were bled for brucellosis evaluation.

**Vaccination**

The Brucella Strain 19 calf elk vaccination program achieved fairly high coverage rates for a relatively mild winter. A total of 2,333 calves were vaccinated on 18 state feedgrounds and the National Elk Refuge during winter 2010. An average of 83% of calf elk were inoculated. Since the inception of the Strain 19 program in 1985, over 86,000 elk have been vaccinated on state feedgrounds and the National Elk Refuge.

**Research**

Totals of 76 vaginal implant transmitters (VITs), 81 GPS collars, and 53 proximity-data logging collars were deployed on elk captured from 11 different feedgrounds and 3 native winter range sites. Of the 44 VITs deployed in elk captured from feedgrounds, 16 of the pregnant cows were determined seropositive for brucellosis. Two of these animals have aborted to date in 2010, both from Dell Creek feedground; one VIT was culture positive for B. abortus and culture is underway on the other. Other research endeavors included: mock-aborted elk fetus contact study using remote cameras and base station proximity loggers, and the Target feedground project.

**TB/Brucellosis Working Group**

Dr. Bill Barton, Idaho State Veterinarian, Brucellosis Committee Vice-Chair

Dr. Barton serves as a member of the Tb/Brucellosis working group. He reported on the working group’s efforts and concurred with Dr. Carter’s remarks regarding the progress of the working group.

**Bull Bison Study**

Dr. Brian McCluskey, USDA-APHIS-VS

A summary of this presentation is included at the end of this report.

**Consortium for the Advancement of Brucellosis Science**

Dr. Walt Cook, University of Wyoming

The Consortium for the Advancement of Brucellosis Science, called CABS, consists of a science team, with members from around the United States (including California, Texas, Louisiana, Virginia, Iowa, Wyoming, and Montana), and stakeholder advisory team comprised of leaders from the Federal Government as well as from the 3 states in the GYA. This consortium is designed according to the model provided by the USDA-NIFA CAP grant programs. The mission of CABS is to evaluate current research, identify gaps, secure funding, award research grants on a competitive and transparent basis, and conduct outreach for the advancement of brucellosis science for domestic and wild animals. Research will focus on development of vaccines, and diagnostic tests.

The goal of the CABS is to work toward successful disease control and prevention. This is a collaborative research effort, with stakeholder consensus, and an adaptive research approach with results to be widely disseminated to policy makers, scientists, and stakeholders.

The CABS project has been designed to further the efforts of the Laramie Agenda, a major meeting with the leading scientists from around the world, which took place in Laramie, Wyoming in 2005. This CABS consortium was proposed at that meeting. Development of improved vaccines and tests for elk, bison, and cattle was estimated to cost $40 million or more and take up to 20 years to undertake.

Approximately $1.8 million per year for the next 5 to 10 years is required to initiate the research projects and operations. Brucellosis has cost the USA and producers billions of dollars since eradication efforts began. Despite the fact that this disease remains a national issue for industry and federal agencies, including USDA-
loss in GYA ranches and to evaluate the reproductive success of GYA herds that have utilized adult vaccination in
 Prior to the field trial, a retrospective observational study will be performed to gauge the normal levels of fetal

Observational study:

want to hold up the process any more than necessary.

track of any open cows he sends to market during the study period.

Choosing which pregnant cows get vaccinated: Every other cow coming to the head gate.

The following spring when all the cows are open, we will return to AV the balance of the study cows. If the producer wants to AV the balance of the cows in the fall, we can return at that time also.

We (the study group) should supply plenty of help when the cows are run through the chute. We don't want to hold up the process any more than necessary.

Observational study:

Prior to the field trial, a retrospective observational study will be performed to gauge the normal levels of fetal loss in GYA ranches and to evaluate the reproductive success of GYA herds that have utilized adult vaccination in
the past. Dr. Kammy Johnson will head this group (Johnson, Dufficy, and Tinker). Dr Dave Dargatz will be the liaison at CEAH for the retrospective group. A survey will be designed and applied to ranches that have had pregnant cows AV boostered, non-pregnant cows AV boostered, and a certain number of operations that have not had their cows vaccinated beyond OCV.

**Vaccine Challenge Studies – Update on RB51 Efficacy in Bison**

Dr. Steve Olsen, USDA-APHIS-VS

A summary of this presentation is included at the end of this report.

**B. suis Diagnostic Research at INL**

Dr. Frank Roberto, Idaho National Laboratory

The presentation, in its entirety, is included at the end of this report.

Dr. Roberto presented on the new genetic testing capabilities that the lab has that can show gaps in the genetic sequence and differentiate between *B. abortus*, *B. melitensis*, and *B. suis*. These new capabilities also allow for the identification of over 100 strains of Brucellosis.

**GYA Wildlife and Livestock Discussion Panel**

Dr. Bill Barton, Moderator, Idaho State Department of Agriculture

Panelists: Dr. Jim Logan, Wyoming Livestock Board

Dr. Eric Liska, Montana Department of Livestock

Dr. Mark Drew, Idaho Department of Fish and Game

Dr. Neil Anderson, Montana Department of Game, Fish and Parks

Dr. Brian McCluskey, USDA APHIS VS

Dr. Brent Schumacher, University of Wyoming

A variety of questions were posed to the panelists addressing the wildlife-livestock interface in the Greater Yellowstone Area (GYA). The panelists were queried as to what tools or procedures were currently being used to address the disease in wildlife within the GYA. The respondents indicated that the three (3) GYA state wildlife agencies are faced with differing circumstances regarding management of wildlife i.e; elk. For instance, Wyoming has established elk feed grounds while Idaho and Montana do not. Idaho continues to endeavor to provide improved and new winter habitat areas for elk in order to prevent depredation on haystacks within the Designated Surveillance Area (DSA) and commingling of elk with domestic livestock on winter livestock feeding areas. Montana has an interest in decreasing herd size and density in an effort to decrease interaction between wild elk and livestock. All three (3) states continue to utilize fencing of haystacks and other feed storage sites coupled with hazing of elk away from livestock operations in an attempt to minimize elk/cattle interaction. Discussion of the established elk feed grounds in Wyoming ensued. Limiting the wild elk feeding period as well as decreasing the concentration of elk on the feed grounds were mentioned as potential mitigation activities to reduce transmission of disease among the elk. It was noted that simply decreasing the number of elk on a feed ground may not necessarily reduce seroprevalence within the herd.

The panel then addressed mitigation activities currently being taken by the livestock industries in the GYA. All three (3) states utilize voluntary herd plans for cattle producers located within the DSA to outline best management practices specific to each operation that serve to mitigate the risk of disease transmission from wildlife to livestock. The use of Official Calfhood Vaccination (OCV), adult booster vaccination with RB-51, fencing haystacks and cattle winter feeding areas, utilization of EQIP funding to establish new water sources for elk and improving wildlife habitat are activities that continue to be utilized.

The question was posed whether management by regulatory agencies of cases of brucellosis in cattle that are determined to be of wildlife origin should be different than herds that have acquired the disease through cattle to cattle transmission. The panelists responded that the two scenarios would not necessarily be handled differently, but that each case should be handled on a case by case basis. Should cattle to cattle transmission be documented, it would be the states responsibility to institute measures to prevent transmission from occurring. It was noted that the genotyping of brucella strains obtained from infected cattle is becoming increasingly efficient at determining the likely source of infection. States should continue to pursue the determination of the likely source of infection in all cases of brucellosis in livestock and take the appropriate steps to mitigate risks associated with transmission of disease.

The panel was queried regarding the most efficacious age to adult booster vaccinate female cattle and what interval should be utilized between booster vaccinations. Dr. Steve Olsen responded that immunity in calves calfhood vaccinated with RB-51 appears to wane at about five (5) to six (6) years of age and that booster vaccination should be done about every three (3) years and at the latest by five (5) years of age.

A delegation of researchers from Russia attended the meeting and made a few comments. The delegation indicated that it appeared the United States had not made significant progress in eradicating...
brucellosis in livestock since their last visit to the U.S. in 2005. They suggested that increased regulations should be placed on landowners and if elk/cattle interaction occurs, livestock owners should be encouraged to move their cattle to a location where infected wildlife are not known to be present. They noted that RB-51 vaccine is not used in Russia as they deem “R forms” of vaccine to be non-efficacious at preventing disease. In Russia Strain 82 has been used for many years and in their opinion that vaccine has been very efficacious in eliminating the disease.

A panel member responded by stating that the U.S. has indeed made significant progress in eliminating brucellosis in livestock and that we continue our efforts to address the issue in the wildlife reservoir in and around the GYA.

Feral Swine Discussion Panel
Dr. Tony Frazier, Moderator, Alabama State Veterinarian

During the second session of the 2010 USAHA Committee on Brucellosis, Nov. 16, a panel dialogue was held to discuss the prevalence of swine brucellosis (B. suis) in feral swine and the potential to spread to livestock. There are similar and dissimilar issues in relation to brucellosis in the GYA but the nature of feral swine and the known spread to cattle along with the zoonotic characteristics make this a point of concern.

The panel was organized by Dr. Jim Logan, chair of the committee on brucellosis and included Dr. Carter Black, Dr. Joe Corn, Dr. Troy Bigelow, Dr. Steve Olsen, Dr. Tom Gidlewski and moderated by Dr. Tony Frazier. Several questions were submitted to the panel with the following remarks.

Current mapping of feral swine distribution by state and federal wildlife agencies report 37 states now have feral swine and this will not be static. These maps demonstrate established populations and clear evidence of breeding. Samples have been collected from 18 states and reveal an 8.4% prevalence of B. suis in feral swine. These animals move about and readily adapt to the environment surviving on whatever food source is available. In addition recent interest in hunting feral swine has increased movement by hunters. There is ongoing work on a vaccine but the limiting factor is a repeatable challenge to assess efficacy. Education and outreach to producers is an important control measure with emphasis on biosecurity. Brucella suis will infect cattle causing the cattle to react to surveillance testing and represents a public health threat where consumption of raw milk is practiced. Members of the panel expressed concern over lack of funding for the continued use of Brucellosis Ring Test (BRT) that could detect B. suis. The response from USDA/APHIS/VS was that the BRT could be used by states but there were no funds to apply. Feral swine also cause extensive property and crop damage in many states but there are no mitigation funds for land owners. There is some work being done by USDA/APHIS/WS in trying to develop a contraceptive using GNRH but delivery systems limit success at this time.

B. suis in Cattle in Texas
Dr. Greg Hawkins, Texas Animal Health Commission

Texas has detected swine brucellosis in 46 cattle in 31 herds since 1998. This presentation will cover the distribution of the B suis cattle in Texas and their relationship to known positive feral swine populations. The presentation will include the methods of detection, and the array of tests utilized to identify and diagnose B. suis in affected cattle. A battery of tests and careful evaluation of each is needed to in order to identify suspect animals from which milk and culture tissues must be collected.

While it is known that infected swine can transmit B suis to cattle, the multiple reactor herds in Texas raises the possibility of cow-to-cow transmission. With the expected curtailment of first-point testing in the U.S., additional research is needed to develop a cattle test specific for B suis, to avoid unnecessary herd testing. Additional research is needed to determine if a latent infection syndrome exists for B suis in cattle, to develop a protective vaccine for cattle, and to ascertain the bacteremic phase of the disease in order to ensure safety of personnel in slaughter plants.

Committee Business:
Three resolutions were brought before the committee for discussion: 1) Winter Feeding of Elk in the Greater Yellowstone Area, 2) Cervid Serology, and 3) Indemnity Funding. All resolutions passed unanimously.
Action items:
It was decided to postpone adoption of the Herd Depopulation Matrix until a later date. The Committee decided that it would be prudent to refine it further and to work with APHIS.

The Scientific Advisory Committee was tasked with evaluating standardization of elk serology in diagnostic interpretation values, and to formulate a white paper on research on infected cattle.
SCIENTIFIC ADVISORY SUBCOMMITTEE REPORT
Dr. Phil Elzer, Committee Chair

Members present: Don Davis TX, Steve Olsen IA, Val Ragan MD, Walt Cook WY, and Phil Elzer LA.
Members absent: Gerhardt Schurig VA, Jack Rhyan CO, Barb Martin IA, Don Evans KS

Introduction of new members:
Dr. Elzer first thanked Gerhardt Schurig and Barb Martin for their years of service and during their tenure with the committee they were instrumental in facilitating the use and standardization of numerous diagnostic assays and vaccines.

Dr. Elzer welcomed Val Ragan and Walt Cook hoping their areas of expertise in regulatory medicine and vaccinology will assist with the pressing issues of the transmission of the disease from wildlife to domestic animals.

70 visitors from various countries, industry, federal, state, etc attended the joint meeting.
There were no formal charges this year but we had numerous discussion points.

Point # 1.
This year the committee met with the Subcommittee on Brucellosis in the GYA since the meetings were running concurrently which prevented all of us from attending the swine subcommittee meeting. It was suggested that next year the three subcommittee meetings do NOT overlap.

Point # 2.
The membership was reminded that if they had any specific items that the committee should look at they need to go through Dr. Logan so he can officially charge the committee. These requests can come from APHIS, industry, individuals, or the other subcommittees. This can be done any time of the year since the committee can and has met quarterly via email or conference call. Items in the past have been approved prior to the annual USAHA meeting.

Point #3.
Select agent status of *Brucella abortus*? No one is exactly sure what is happening with delisting *Brucella abortus* from the select agent list. It seems that some headway is being made but there still might be some associated security issues which might delay any progress. Please keep this issue in the forefront because it is critical that *Brucella abortus* is removed so research into new vaccines for bison and elk can resume. Note the groups representing human and animal health voted to delist *Brucella abortus* but Homeland Security still has concerns.

Point # 4.
The committee welcomed the delegation from Russia. These scientists are presenting their brucellosis vaccine data in the biologics session on Monday evening.

Point # 5.
There was a lengthy discussion on what to do with animals ie cattle which become infected from wildlife reservoirs. Is it wise to kill the infected animal? Are we killing the diagnosis? If the animal has offspring should they be killed? Can we learn anything from any of these animals or their offspring? Can we learn about latent heifer syndrome, genetic make up of herd, is anything novel going on, etc?

It was suggested that a white paper be formulated to include the following: Project narrative, expected outcomes, budget, facilities, etc. The purpose of the paper should focus on the elimination of disease and decrease prevalence in wildlife and domestic animals.

Dr. Logan charged the committee to have a draft of the white paper finished by next quarter.

The committee will be putting forth a resolution to update the Brucellosis in Cervidae Uniform Methods and Rules to include all brucellosis serological tests and cutoffs for cervids.
FERAL SWINE SUBCOMMITTEE REPORT
Dr. Carter Black, Chair

The Subcommittee met on Sunday, November 14, 2010. Forty three persons were in attendance with eight committee members at the meeting. Reports were provided on a number of feral swine issues of interest to USAHA and its members. A summary of the reports is included below.

Dr. Joseph L. Corn, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, provided an update on the National Feral Swine Mapping System (NFSMS). SCWDS produced nationwide feral swine distribution maps in 1982, 1988 and 2004 by working directly with state and territorial natural resources agency personnel. In 1982, 17 states reported feral swine in a total of 475 counties. In 2004, 28 states reported feral swine in 1014 counties. With support from USDA-APHIS-Veterinary Services (VS) the SCWDS recently developed the National Feral Swine Mapping System (NFSMS), an interactive data collection system used to collect and display real time data on the distribution of feral swine in the United States. The real time feral swine distribution maps are produced using data collected from state and territorial natural resources agency personnel and from USDA-APHIS-Wildlife Services (WS). The real time map is available to be viewed by the public on the NFSMS home page. Distribution data submitted by agency personnel are evaluated by SCWDS on a continual basis, and the real time distribution map updated with verified additions on a monthly basis. Feral swine populations and/or sightings are designated either as established and breeding populations, or as sightings, but only established breeding populations are included on the map and in the total of the number of states with feral swine. Currently 37 states are reporting established feral swine populations. Over 450 additions have been made to the feral swine distribution map through the NFSMS since January 2008. The NFSMS is accessed via the internet at http://www.feralswinemap.org/.

Dr. Troy Bigelow, USDA-APHIS-VS reported on the status of brucellosis and pseudorabies in the country. The surveillance system is primarily slaughter samples. USDA-APHIS-WS is conducting CSF surveillance on feral swine with a total of 2,395 samples collected. Education of swine producers of the risk of feral swine will help to reduce the chances of introduction of brucellosis and pseudorabies into the domestic herd.

Dr. Thomas Gidlewski, USDA- APHIS-Wildlife Services-National Wildlife Disease Program, gave an update on the Comprehensive Feral Swine Disease Surveillance and Monitoring Program. The National Wildlife Disease Program (NWDP) conducts the Comprehensive Feral Swine Disease Surveillance and Monitoring Program in an attempt to detect foreign animal diseases (FADS) as well as to monitor the status of endemic diseases. Feral swine may serve as reservoirs for many endemic diseases such as swine brucellosis (SB) and pseudorabies (PR), and act as a high risk pathway for the introduction of these and other diseases into the commercial livestock industry. Forty-nine states are currently free of brucellosis in commercial swine, however SB is endemic in the feral swine population and occasionally spills over into transitional herds. In FY2010 the apparent prevalence of SB was 3.4% nationally. Pseudorabies virus (PRV) causes disease in swine, but can also infect cattle, sheep, goats, and many species of wildlife. The virus was officially eradicated from the commercial swine industry in 2004, but like SB, remains endemic in feral swine and is found occasionally in transitional herds. PRV occurred at an apparent prevalence of 15.4% nationally in FY2010. The comprehensive feral swine disease surveillance and monitoring program takes advantage of the 30,000 feral swine that are removed annually nationwide by feral swine damage management activities conducted by Wildlife Services. The program began in 20 states in FY2007. In FY 2011 wildlife disease biologists in 35 states are expected to collect serum and tissues from over 3000 feral swine. Diseases monitored this year will include swine brucellosis, pseudorabies, swine influenza, porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type-2 infection, toxoplasmosis and trichinosis. The program will continue to conduct surveillance for classical swine fever (CSF) as well as negative cohort sampling for African swine fever (ASF) and foot-and-mouth disease (FMD). Additional projects include maintenance of a feral swine serum archive for retrospective study, a feral swine brucellosis tissue culture study, genotyping of Trichinella and Toxoplasma extracted from tissues, and a pilot tuberculosis (TB) monitoring project. The program emphasizes sample collection in areas previously not sampled or under-sampled and areas that contain new populations, but continues to monitor areas previously sampled. The goal is to provide information on the long-term persistence of certain diseases such as SB and PR and monitor the feral swine populations for foreign and emerging diseases in different states and regions.

Dr. Steven Olsen, National Animal Disease Center reported on the evaluation of a rough B. suis vaccine strain in swine. In recent years we have conducted multiple projects evaluating the safety, immunogenicity, and efficacy of a rough B. suis strain (strain 353-1) that was isolated from a feral swine herd in North Carolina. Parenteral vaccination of swine with 2 x 10^10 CFU of 353-1 induces antibody responses that peak around 2 to 3 weeks after vaccination and persist for up to 29 weeks. Oral vaccination with 10^{11} CFU of 353-1 induced a similar profile although mean antibody titers were lower when compared to parenteral vaccination. Strong cell-mediated responses were noted after oral or parenteral vaccination, beginning as early as 3 weeks and detectable for as...
effectively delivered orally, it may be a tool to help manage the high prevalence of brucellosis in feral swine.

Non-vaccinated swine co-housed with vaccinates did not seroconvert and the vaccine strain could not be isolated from samples obtained at necropsy. This data suggest the vaccine is safe as it is not transmitted laterally.

Vaccinates also demonstrated greater protection against experimental challenge with a virulent B. suis biovar 1 strain. Colonization (CFU/gm) in target tissues was reduced in vaccinates when compared to control swine.

Our data suggests that 353-1 is a safe and efficacious vaccine for swine. As it is a natural mutant and can be effectively delivered orally, it may be a tool to help manage the high prevalence of brucellosis in feral swine.

Drs. Harold R. Garner1, Shamira J. Shallom1, Luciana Sarmento2, Dale Preston3, Christopher Franck1, and L. Garry Adams4 (1Virginia Bioinformatics Institute and Department of Statistics, Virginia Tech, Blacksburg, VA 24061; 2Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station TX 77843-4467; 3Texas Animal Health Commission, State-Federal Diagnostic Laboratory, Austin, TX 78723) gave an update on Brucella spp. Microarray Detection and Phylogenetic Classification: Universal Biosignature Diagnostic Assay (UBDA) Technology for Known Brucella spp. and Unknown Near-Neighbors Isolated from Feral and Domestic Livestock. Genomic DNA samples (40 samples) were received from Texas Animal Health Commission (TAHC) and Texas A&M University. These DNAs samples were prepared from Brucella abortus and Brucella suis organisms cultured from milk samples collected from bovine brucellosis suspects based on the antibody-based diagnostic tests. The organisms were heat inactivated and treated with methanol and genomic DNA extractions were performed at Texas A&M University.

Of the 40 TAHC genomic DNAs, 29 samples had genomic DNA concentrations greater than 5 ng/ul. Nine of these TAHC samples were hybridized on the Universal Biosignature Diagnostic Assay (UBDA) array that is a species independent array comprising mainly of 45 probes (262,144) which are computationally derived and genome independent. The microarray contains probes that are tailored to be genome independent, pathogen and bacteria specific, and detect microsatellites, antibiotic resistance genes, and control probes. This unique strategy uses the robustness of patterns generated from hybridization of any unknown genome (DNA or cDNA) to a very high-density species independent oligo-nucleotide microarray. Hybridization patterns could be unique to a genome, and potentially to different isolates and to a mixture of organisms. Different genomic DNA samples were labeled with Cy3 or Cy5 and hybridized on the UBDA array. Data files were background subtracted and quantile normalization. A parsing script written in PERL was used to extract probes related to the randomer (262,144 probes) from the 354K array. Hierarchical clustering (Eisen et al. 1998) transforms a distance matrix of pair-wise similarity measurements between all items into a hierarchy of nested groupings. The hierarchy is represented with a binary tree-like dendogram. Fourteen data files were clustered including 9 samples received from TAHC and compared with standard Brucella melitensis 16M, Brucella abortus 12, Brucella abortus 86-8-59, Brucella abortus RB51, and Brucella suis 1330 from BEI resources. The clustering algorithm revealed that three of the TAHC biochemically phenotyped B. suis failed to cluster with the standard B. suis 1330, instead clustered as having both B. suis and B. abortus genomic DNA, or an unknown intermediate genotype. TAHC samples were further analyzed by PCR using 25 ng of starting material, using primer sets chosen for Brucella abortus and Brucella suis (Bricker et al. 1994) which confirmed that these samples contained either both B. suis and B. abortus, or an unknown intermediate genotype. These preliminary UBDA data will be presented and discussed for biological relevance and potential application to further understand the infection biology of Brucella spp. in the epidemiology brucellosis of feral and domestic animals.

Dr. Kurt VerCauteren, Michael Lavelle, Justin Fischer, and Greg Phillips, United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado; Trevor Hefley and Scott Hygnstrom, School of Natural Resources, University of Nebraska, Lincoln, Nebraska; Seth Swafford, United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Disease Program, Fort Collins, Colorado; and David Long and Tyler Campbell, United States Department of Agriculture, Animal and Plant Health Inspection Services, Wildlife Services, National Wildlife Research Center, Texas Field Station, Kingsville, Texas; provided an update on containment of feral swine under simulated depopulation conditions. Feral swine impact property, crops, and livestock in the US, possibly in excess of a billion dollars annually, and also have potential to spread many diseases. Effective and quick-to-erect means for containing feral pigs (Sus scrofa) are needed in the event of a catastrophic disease outbreak. We considered 5 candidate fence types and based on efficacy, selected traditional 0.86-m high hog panels for containing wild-caught feral swine within 2 test enclosures through multiple trials under various levels of motivation in southern Texas. Fences proved 97% successful under minimal motivation without pursuit, 83% effective when feral swine were pursued by walking humans discharging paintball projectors, and 100% successful when feral swine were pursued and removed by gunners in a helicopter. All feral swine that escaped did so going over the fence, so a taller fence may eliminate virtually all breaches.

long as 29 weeks after vaccination. Peripheral blood mononuclear cells also produced significant amounts of interferon-gamma in vitro in response to incubation with Brucella antigens.
Constructing fences of hog panels resulted in effective, relatively inexpensive ($5.73/m), and easy to erect enclosures.

Dr. Stephanie A. Shwiff, Tyler Cozzens, Mark Lutman, and Seth Swafford, USDA-APHIS-Wildlife Services-National Wildlife Research Center, Fort Collins, Colorado, provided an update on the Economic Benefit of Feral Swine Disease Surveillance: Foot and Mouth Disease. Feral swine are a known reservoir and vector for economically threatening diseases. In the late 1980's, the distribution of feral swine was found mostly in southern states. Due to their high fecundity, mobility and adaptability, feral swine populations are rapidly expanding throughout the U.S. Combined with their frequent interactions with livestock and people, feral swine are quickly increasing the risk of endemic disease transmission, as well as the potential spread of foreign animal diseases such as foot-and-mouth disease (FMD). The economic impact of FMD introductions into livestock operations was shown by the dramatic losses suffered in Taiwan ($1.6 billion in 1997) and the U.K. ($11 billion in 2001). Early detection and monitoring of FMD through surveillance can be an effective way to minimize the spread, therefore minimizing the resulting impacts to the livestock industry and the economy. Disease surveillance in feral swine is currently being conducted across the U.S. by Wildlife Services National Wildlife Disease Program (NWDP) to monitor for several important diseases (e.g., pseudorabies and swine brucellosis). For this study, the economic benefit of surveillance was estimated as the decrease in the potential negative economic impacts caused by feral swine transmitting FMD in California, Kansas, Iowa, Missouri, North Carolina, and Wisconsin. To estimate this benefit, we simulated a hypothetical FMD outbreak with different levels of feral swine disease surveillance using a bioeconomic model. Initial results of this simulation indicate that surveillance significantly reduced the potential negative economic impacts of FMD. This presentation will discuss the bioeconomic model used and our initial results, using North Carolina as a case study.

There was a discussion of a previous resolution from the committee which related to B. suis infection in cattle. The work that Dr Olsen reported on was the scope of work needed to resolve this problem. With no other discussion, the committee adjourned at 4:30 PM.
The subcommittee met on November 14, 2010 with subcommittee chair, Marty Zaluski, calling the meeting to order at 12:30 PM. The subcommittee meeting was held in conjunction with the Scientific Advisory Subcommittee.


The subcommittee received several presentations.

Dr. Logan, Wyoming state veterinarian discussed some circumstances of the 2010 brucellosis affected herd. The herd, located in Meeteetse, WY has reported frequent co-mingling events between cattle and wild elk. The elk are not known to be associated with a feedground.

Neil Anderson, from Montana Fish Wildlife and Parks, presented on trend in brucellosis prevalence in Montana elk, and described a multi-year, elk live capture study that will commence in early 2011.

Mark Drew, biologist for the Idaho Department of Fish and Game discussed prevalence of brucellosis in Idaho elk, and the policies on winter feeding. Mark drew stated that the prevalence of brucellosis in Idaho elk does not seem to be increasing from the 2% known to be infected.

The subcommittee discussed and voted on the resolution addressing winter feeding of wild ungulates in the GYA regarding brucellosis transmission. The resolution passed by majority vote.

The subcommittee meeting adjourned at approximately 3:30 PM.
Brucellosis Eradication Program

State Status Classification: October 1, 2010

Class A Status
Free Current Year
Free 1-5 yrs
Free 1-10 yrs
Free 6-10 yrs
Free 11-15 yrs
Free 16-20 yrs
Free 21+ yrs

Texas was officially classified as Brucellosis Class Free on February 1, 2008.

Most Recent Activities Impacting State’s Status (as of October 1, 2010)

- Idaho: One brucellosis-affected herd was disclosed in December 2009. An affected herd plan is in place which includes herd testing and depopulation activities. The epi-investigation has been completed. No additional affected herds were disclosed. Class Free State status has been maintained.

- Texas was officially classified as Brucellosis Class Free on February 1, 2008.
National Prevalence Rate: Brucellosis Affected Cattle Herds

FY 2004 = 0.0005%  FY 2006 = 0.0002%  FY 2008 = 0.0003%  FY 2009 = 0.0000%  FY 2010 = 0.00001%

FY 2005 = 0.0003%  FY 2007 = 0.0001%  (as of October 1, 2010)

MCI Surveillance:
- Approximately 6.170 million head of cattle tested
- Approximately 400 MCI suspicious test results
  - The single brucellosis-affected cattle herd disclosed in FY 2010 was disclosed through slaughter surveillance testing
  - All other MCI suspicious test epi-investigations confirmed negative herds

BRT Surveillance:
- Approximately 114,620 BRTs conducted on 53,540 commercial dairy herds
- Approximately 77 suspicious BMST results
  - All BRT suspicious epi-investigations confirmed negative dairy herds

On-Farm testing:
- Approximately 486,000 additional head of cattle were tested on-farm
  - Reason for testing:
    - movement and sale (~33%)
    - herd certification (~27%)
    - epidemiologic investigations (~20%)
    - show/exhibition purposes (~10%)

Calfhood Vaccination:
- Approximately 3.10 million calves were vaccinated
  - Brucellosis certified-free herds:
    - Approximately 2200 brucellosis certified-free cattle herds

*Note: These statistics reflect FY 2010 data reported as of October 1, 2010*

The Brucellosis Concept Paper was published in the Federal Register on October 5, 2010 and received a total of 361 comments. Action Plan Proposed Components include:
- Demonstrate the disease-free status of the U. S.
- Mitigate disease transmission from wildlife
- Enhance disease response and control measures
- Modernize the regulatory framework
- Implement a risk-based disease management area concept

Key comments included:
- Effectively demonstrate national disease-free status
  - Developing a National Surveillance Strategy
  - Shift from a State-by-State surveillance system to a national surveillance strategy
  - Consolidate Surveillance Laboratories and Use Standardized Protocols
• Enhance Efforts to Mitigate Disease Transmission from Wildlife
  o Potential strategies include:
    ▪ Partnering with State and Federal wildlife agencies to conduct wildlife surveillance
    ▪ Developing on-farm mitigations to control disease-transmission risks between wildlife and livestock
    ▪ Supporting research to find tools (e.g., vaccination and contraceptives) and strategies (e.g., habitat management) to reduce the prevalence of brucellosis in wildlife

• Enhance Disease Response and Control Measures
  o Define prevalence on a “case” basis
  o Develop alternative strategies to depopulation
  o Official animal ID & electronic movement certificates

• Modernize the Regulatory Framework
  o More flexible rulemaking is needed to address disease situations based on risk
    ▪ Quick response to changing program needs
    ▪ Employ up-to-date science
    ▪ Flexible enough to adapt to unique and varying disease situations

• Risk-Based Disease Management Areas
  o Facilitate disease risk mitigation
    ▪ Designated surveillance areas in the GYA
  o Provide confidence in the United States’ disease-free designation
  o Collaborative State-Federal effort

• There is Overall general support
• Many comments addressed specific issues and/or concerns
  o GYA issues
    ▪ Wildlife
    ▪ Mitigation measures
  o Designated surveillance areas
  o Funding and effects on states

Status of the Campaign Against Brucellosis in Mexico
Jose Alfredo Gutierrez
CGRPA, Mexico

Health and Food Safety in Mexico.
197 million tons of food produced.
• Inventory at large livestock (cattle, pigs, birds, horses, sheep, goats and beehives).
• 17 billion dollars of food exports.
• The competitiveness of agriculture, livestock, aquaculture and fisheries in Mexico.

Budget BR Eradication Program
• Cattle
  - 2009 FY: $62,646,964
  - 2010 FY: $53,733,639
• Caprine and Ovine
  - 2009: FY $25,094,539
  - 2010 FY: $18,922,328

Fig. 1 Current Classification Status for National Brucellosis of Mexico - 2010
### National Diagnostic Tests

#### BOVINE

<table>
<thead>
<tr>
<th>YEAR</th>
<th>TESTS</th>
<th>POSITIVES</th>
<th>FREQUENCY (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HERDS</td>
<td>HEADS</td>
<td>HERDS</td>
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<tr>
<td>2009</td>
<td>136,710</td>
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<tr>
<td>2010 *</td>
<td>83,587</td>
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#### SHEEP

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<th>FREQUENCY (%)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>HEADS</td>
<td>HERDS</td>
</tr>
<tr>
<td>2009</td>
<td>22,239</td>
<td>598,168</td>
<td>177</td>
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<tr>
<td>2010 *</td>
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<td>411,589</td>
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#### GOATS

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<th>POSITIVES</th>
<th>FREQUENCY (%)</th>
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<tr>
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<td>HERDS</td>
</tr>
<tr>
<td>2009</td>
<td>6,327</td>
<td>261,715</td>
<td>418</td>
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<tr>
<td>2010 *</td>
<td>6,477</td>
<td>231,803</td>
<td>384</td>
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Fig. 2 National Diagnostic Tests

**Fig. 3. National Brucellosis Vaccination Report**
Fig. 4. Mexico’s Accredited Brucellosis Free Herds

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<th>SHEEPS</th>
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<td>HERDS</td>
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Table 1: Incidence of Human Brucellosis in Mexico 2009-2010

<table>
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<th>Year</th>
<th>Cases</th>
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<tr>
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<td>5,487</td>
</tr>
<tr>
<td>2010</td>
<td>2,928</td>
</tr>
</tbody>
</table>

An Electronic System for Decentralization of the Issue of Certificates of Free Brucellosis Herd has been implemented. The procedure for obtaining certificates of free herd has been reduced from four months to 15 days.

Fig. 5 Incidence of Human Brucellosis in Mexico

*update September/30th/2010. Week 33. EPIDEMIOLOGY GENERAL DIRECTION/PUBLIC HEALTH MINISTRY*

The Strategic Monitoring Plan includes:
- Vaccination program in regions with goat & bovine high or middle prevalence.
- Infected Dairy herd management by brucellosis.
- Increase in the epidemiological surveillance in slaughterhouses.
- Marking and elimination of positive animals.
- Coordination between SENASICA-COFEPRIS, in order to exchange useful information focused on decreasing the disease in the human population.
- Improving the coverage of vaccination and promote the Certificate of free herds.

Fig. 6. Expected 2010 Status
Project Objectives included:
- Develop a process to evaluate the presence of *B. abortus* or risk of introduction
- Provide standardized, scientific approach to decision making
- Improve transparency
- Be consistent with the proposed changes to the Brucellosis program

The World Organization for Animal Health (OIE) defines a zone/region as “a clearly defined part of a territory containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control, and biosecurity measures have been applied for the purpose of international trade.”

What to Consider:
- Is the pathogen contained in the livestock population in the region?
- Are all pathways for pathogen spread contained in the region?
- Is risk of pathogen leaving region sufficiently mitigated?

Evaluation of a region for import
- 2 or more years
- Country requests import
- Hazard ID
- Information is collected to evaluate 11 risk factors (9CFR 92.2)
- Risk assessment conducted
- Trade negotiations with risk managers

Evaluation of regions domestically
- Import assessment process too slow and not always applicable
- Data from States is varied, difficult to enforce
- Inconsistent methods applied

Management Area Evaluation Process
1. States designate a management area (MA)
2. Fill out an application
3. Model is run
4. MOU created with State

Fig. 1 Management Area Evaluation Process
Step 2: Fill out the Application
- What surveillance has been done?
- What do you know about the risks?
- How are you controlling risk?
- How will you manage the area?

Step 3: Run the model
For Each Geographic Unit
- What is the probability that *B. abortus* is present?
  - Prevalence/surveillance
- What is the probability that *B. abortus* will be introduced
  - Via wild elk or bison
  - Via cattle co-grazing or new additions

Model structure

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Step 3- Map Model results
Step 4: MOU Development

- Evaluate why risk is high
- Evaluate alternate mitigations
- Evaluate management of entire State-
  - Resources, etc
- Re-evaluate every 1-2 years?
- Advantages and disadvantages
- Disadvantage
  - Inflexible for new pathways and pathogens
  - Limited by spatial scale State provides
  - Limited by lack of data
- Advantage
  - Rapid
  - Transparent
  - Consistent
  - Relative risk comparison
  - Minimal data needed

The next steps for the project will include the continued validation of the model. We will identify criteria for management answers and test with realistic scenarios. We also plan to automate the application procedure.

Study of Shedding and Venereal Transmission of *Brucella abortus* by Bison Bulls in the Greater Yellowstone Area

Brian McCluskey
USDA-APHIS-VS

Purpose

- Investigating proposals to eliminate brucellosis from Yellowstone bison using nonlethal strategies.
- If venereal transmission from bulls to cows occurs, even in a minority of breedings, then strategies relying only on preventing shedding by bison cows would be ineffective.
- Strategies to remotely vaccinate bison, especially if extended to adult vaccination, could be tailored to include or not include bulls depending on results of venereal transmission studies.
- Under IBMP adaptive management strategy, year round access for bulls to public lands in Montana is desired –lead to bison bulls in closer proximity and possibly commingling with cattle grazing in the area
- Question of bison bulls located out of YNP shedding *B. abortus* has been posed. This study would be useful in determining the risk that infected bulls might pose to cattle in proximity to bison.

The Project was done in Two Phases

**Phase One:**
- Time: Spring 2010, Spring 2011
Phase Two:
- Area: West and North of YNP
- Time: Summer/Fall 2011, Summer/Fall 2012
- Area: TBD

The Study Area for Phase One included:
- West of Yellowstone National Park area- designated by the Interagency Bison Management Plan (IBMP) as Zone 2.

Capture Objectives
- Perform breeding soundness examinations
- Collect semen and blood samples from individual animals
  - Semen evaluated microscopically for quality
  - Semen cultured for *B. abortus*
  - Serum will tested for *B. abortus* antibodies
- Bulls will be selected that are 2 years of age or older
  - Final goal of 75% of bulls being over 3 yrs of age.

Activity Summary, Spring 2010
- April-May 2010; 39 individual bison bulls were captured and sampled in the IBMP Zone 2 areas in Montana surrounding Yellowstone National Park.
- The age of bulls: from 2 yrs through over 10 years of age
  - 18 > 6 years old
  - 19 were between 3 and 6 years
  - 2 bulls were 2 year olds.
- Body condition:
  - 36 bulls: good or moderate
  - 3 bulls were classified as thin.
- Immobilization times: 16-69 minutes
- The average immobilization time was 26 minutes
- Scrotal circumference: 24 cm-40 cm,
  - Average of 34.5 cm for bulls >over 3
  - Average of 27 cm for bulls 3 years and younger.
- Physical examination: 3 of the 39 bulls
  - evidence of seminal vesiculitis.
- Gross individual sperm motility: Ranged from 0-60%.
- Serologic tests:
  - 25 (64%) were positive for *B. abortus* antibodies
  - 2 were considered suspects
  - Culture results:
    - *Brucella abortus* biovar 1 from 2 bulls:
      - YNP95021 Semen ~1 cfu/ml
      - YNP95023 Semen ~8 cfu/ml
Evaluation of Booster and Dart Vaccination with RB51

- Parenteral vaccination at 8-10 months of age with $10^{10}$ CFU (n=16)
- Booster vaccination of half of parenteral vaccinates with $10^{10}$ CFU 13 months later
- Dart vaccination with $10^{10}$ CFU at 8-10 months of age
- Saline Control
Antibody Responses after initial Vx

Antibody Responses after Booster Vx

Proliferative Responses after Initial Vx
Conclusions
- Booster vaccination at time of breeding did not cause fetal infection or reproductive losses
- Booster vaccination increased efficacy against experimental challenge
- Correlates of protective immunity?

Efficacy of RB51 against *Brucella suis*
- Temporal characterization of serologic responses after *B. suis* infection
- Determine if RB51 vaccination protects against *B. suis* infection

Experimental Design
- Vaccinates: $10^{10}$ CFU of *B. abortus* strain RB51 at 10 months of age
- Saline control group
- Measure immune responses after vaccination
- Challenge in midgestation with $10^7$ CFU of *B. suis* isolates obtained from cattle in TX
# Post-Challenge Serology to *B. suis*

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Regional Wildlife Issues

- What is the real potential for interspecies transmission between large ungulates and cattle in the Intermountain West?
- Can rapid, accurate molecular diagnostics improve management and minimize impacts to wildlife?

Figure 1.

Distribution of the northern (green) and central (yellow) bison herds within Yellowstone National Park. Red indicates seasonal migration outside of the park boundaries. Green triangles indicate sites where samples have been taken for real-time PCR and cultivation analyses.

Real-time PCR assay development

*Figure 2.*
Genome-based Brucella taxonomy
Bohlin et al., 2010. BMC Evol. Biol. 10:249
Figure 3.
Brucella Test Panel

- All type strains except for microti and inopinata
- 60 B. abortus, including 26 recent bison and elk isolates from Montana and Wyoming, S19 and RB51
- 3 human isolates of B. melitensis, and Rev. 1 vaccine strain
- Human, cow, pig, hare field isolates of B. suis
- Ochrobactrum anthropi as near neighbor
- 1 anomaly – WY elk isolate positive with B. suis assay, but NOT with B. abortus assay

Real-time PCR assay for B. abortus


- B. abortus specific (tested against panel of over 100 strains)
- 7.5 fg limit of detection (ca. 2 genomic copies)
- Semi-quantitative nature of real-time PCR permits estimation of bacterial load in samples
- Detection in 15-30 minutes
- No sample prep necessary in some cases
• Hybridization probes allow discrimination of amplicons based on post-amplification melt curves (potential to identify S19 and RB51 without multiplexing)
• New TaqMan assay developed for other instruments (15 fg LOD)

Real-time PCR assay for *B. suis*
• *B. suis* bv.1 (may also detect 2-4, but results inconclusive with only one strain of each available)
• 2 fg limit of detection (less than 1 genomic copy)
• TaqMan assay developed on ABI 7000 and 7900HT Fast block (25μl rxn)
• Detection in 15-30 minutes

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