REPORT OF THE COMMITTEE ON BRUCELLOSIS

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The Committee met on October 22, 2012 at the Greensboro Sheraton Hotel, Greensboro, North Carolina, from 1:00 to 6:00 p.m. There were 28 members and 19 guests present. Introductions of Vice Chairs and Subcommittee Chairs were made. An overview of the 2011 meeting and resolutions were given.

Presentations and Reports

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

Dr. Joe Corn presented the Feral Swine Subcommittee Report, which is included at the end of this report.

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

National Brucellosis Program Update

Dr. Mike Carter
USDA-APHIS-VS

Since July 10, 2009, all 50 States, Puerto Rico, and the US Virgin Islands have been classified as Class Free for bovine brucellosis. During the fiscal year (FY) 2012, national and state surveillance has identified four bovine brucellosis-affected herds; two located in Idaho, one in Montana and one in Wyoming. However, as a result of the interim rule, there was no loss of Class Free State status due to new provisions.

During FY 2012, approximately 3.3 million head of cattle under the Market Cattle Identification (MCI) surveillance program, reflecting approximately 3.3 million head of cattle tested at slaughter and approximately 478,000 head of cattle tested at market. There were approximately 3.9 million calves and approximately 16,420 adult cattle vaccinated for brucellosis and there were approximately 1,100 brucellosis certified-free cattle herds. Approximately 405,000 additional head of cattle and domestic bison were tested as a result of other surveillance activities. Three of the four brucellosis-affected herds disclosed in FY 2012 were disclosed during testing conducted as part of the State's increased surveillance activities. The one cattle herd that was detected outside the Idaho's designated surveillance area was detected through slaughter testing. The primary reasons for testing on-farm or ranch includes testing for movement and sale (~45%), testing associated with MCI reactor investigations and affected herd epidemiologic investigations (~13%), herd certification testing (~23%), and testing for show or exhibition (~6%).

Since the publication of the Brucellosis interim rule in December 2010, the 60-day comment period has ended and thirty comments were received from private citizens, State agencies, industry groups, animal welfare organizations, environmental groups, and Congress. The rule has been designated as significant by the Office of Management and Budget. Additional economic analysis and civil rights impact analysis were completed and in July, APHIS provided additional information to the department regarding the changes reflected in the interim rule. The final rule is currently within the review process.

APHIS continues to develop new regulations and supporting standards for the brucellosis and tuberculosis (TB) programs. Under the proposed approach, The *Code of Federal Regulations* will provide the legal authority for the programs while the details of the programs will be described in a program standards document.

APHIS conducted several webinars that provided additional information about the proposed regulation in FY 2012. APHIS proposed to use a national calculator to determine the fair market value for animals that are destroyed because of TB or brucellosis in the Draft Regulatory Framework published in May 2011. In response to requests from commenters, APHIS hosted two webinars in November 2011 that provided more information about the calculator and options for indemnity payments. The end result from comments received is changes to the indemnity process will not be included in the Proposed Rule. In August 2012, APHIS presented an overview of the Proposed Rule and Program Standards for Brucellosis and Bovine Tuberculosis. The webinar presentation described the fundamental concepts underlying the proposed regulations, the content of both the Proposed Rule and the Program Standards, and significant differences from the draft regulatory framework and the rationale for these differences. Recordings of both webinars are available at: http://www.aphis.usda.gov/animal_health/tb_bruc/webinars.shtml.

USDA, Animal and Plant Health Inspection Service (APHIS) is hopeful that Proposed Rule and Program Standards will be published in Federal Register in early FY2013. Both documents are currently under Agency review. Upon publication, APHIS plans to provide an extended comment period of 90 days through the www.regulations.gov website in light of the scope of these regulations.

In September, APHIS initiated a review of Idaho, Montana and Wyoming with the goal to determine the adequacy of each State's Brucellosis Management Plans in preventing the spread of brucellosis from the designated surveillance area (DSA). The same nine member team visited each state. The review focused on seven key questions that include:

1. Are States adhering to their best management practices (BMPs)?
2. Is privately owned bison and cattle surveillance effective?
3. Are protocols for testing used for epidemiological investigations, test and remove protocols, and quarantine release are documented and being followed?
4. Are adequate regulations in place to prevent the movement of brucellosis-infected cattle or domestic bison out of the DSA and is compliance monitored?
5. Are identification requirements being enforced and are animals traceable to the DSA?
6. Is wildlife surveillance sufficient to allow for rapid adjustment of the boundaries of the DSA?
7. Are mitigations in place that reduce exposure to infected sources and reduce the risk of infection if exposure occurs?

The National Bovine Brucellosis Slaughter Surveillance program is one element of a larger surveillance plan, entitled "National Bovine Brucellosis Surveillance Plan: October 2012." This plan is available on the Animal and Plant Health Inspection Service Web site at www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/. Slaughter surveillance is not the only brucellosis surveillance stream that is and will be evaluated to determine the national brucellosis status. Other surveillance streams — diagnostic, export, movement, and herd certification testing — will also be used to support the claim of US brucellosis freedom. Veterinary Services (VS) is also piloting enhanced passive surveillance projects that could be expanded in areas that need additional surveillance.

In July 2011, VS announced changes to the National Bovine Brucellosis Slaughter Surveillance Program. This included reducing the brucellosis slaughter surveillance samples from approximately 6 million samples to approximately 3 million samples. In 2012, due to growing budget concerns, VS evaluated the program and determined further modifications were needed to our baseline surveillance activities to improve the program's cost effectiveness. The revised goal is to detect *Brucella abortus* infection with a 95 percent confidence that the prevalence level does not exceed one infected animal per 100,000 animals and documenting disease freedom at that level. Blood samples will be collected at eight selected slaughter plants. This strategy provides a statistical sampling of 1 million to 1.2 million slaughter surveillance samples. VS will continue to evaluate the brucellosis surveillance program and will propose further changes to participating plants or number of samples collected if necessary.

The complete presentation is included at the end of this report.

Montana Report Summary

Dr. Marty Zaluski
Montana State Veterinarian

Montana implemented a designated surveillance area (DSA) shortly after reclassification to Brucellosis Class A following the detection of the second affected herd in 2008. Montana's DSA is part of four counties of Beaverhead, Gallatin, Madison, and Park and includes 264 herds that use the area either full time or seasonally. Since 2007, the state has detected a total of five herds (total of four owners) which include three cattle herds and two domestic bison herds. One of the domestic bison herds recently completed a herd test and found nine reactors which brought the total number of Montana reactors since 2007 to 28. The two domestic bison herds under one ownership remain under quarantine in Montana.

A USDA-APHIS-VS review team recently conducted a review of Montana's management plan and made the following findings.

Key strengths of Montana's brucellosis management plan include:

- Proactive actions leading to adjustments to the boundaries of Montana's designated surveillance area;

- Cooperative efforts between Montana Department of Livestock's Animal Health Division and their Brand's Enforcement Division, including the implementation and use of an electronic brands software program at the livestock markets; brand inspection plays a critical role in Montana's brucellosis management plan;
- Wildlife surveillance activities, most notably the multiyear elk capture and surveillance project;
- Testing and surveillance requirements for domestic cattle and bison in the designated surveillance area; and
- Use of individual herd plans for herds located in the designated surveillance area.

Key recommended enhancements to Montana's brucellosis management plan include:

- Increasing the number of herds within the designated surveillance area on approved herd plans. Risk assessments should be conducted on each herd prior to developing an individualized herd plan.
- Developing a template for a formal brucellosis-affected herd plan and a template for approved designated surveillance area herd plans detailing the proactive risk mitigation actions in place.
- Increasing surveillance on slaughter cattle coming out of the designated area, especially when going direct to slaughter.
- Continuing wildlife surveillance activities and studies to expand the knowledge base about brucellosis in elk which in turn will lead to better disease management practices and risk mitigation efforts.
- Working with APHIS to develop a state-specific (or designated surveillance area specific) slaughter cattle surveillance plan (sampling and testing pre-slaughter).
- Continuing producer education and outreach using a variety of venues through which to deliver and disseminate information about Montana's brucellosis surveillance program.

Idaho Report Summary

Dr. Bill Barton
Idaho State Veterinarian

The 2009 Idaho affected herd was released from quarantine on March 2, 2012. Idaho changed its Brucellosis rules during the 2012 legislature. The following changes were made to the rules:

- Mandatory official identification is required on all sexually intact cattle, regardless of age, that spend time in Idaho's DSA.
- All sexually intact cattle, 18 months of age or older, that have been in Idaho's DSA between January 1 and June 15 of the calendar year are required to be tested for brucellosis within 30 days prior to change of ownership, interstate movement or movement outside of the DSA.

Idaho State Department of Agriculture (ISDA) identified two new brucellosis affected herds in early 2012:

- A domestic bison herd tested due to known elk/bison interaction identified two reactor animals.
  - Both were slaughtered and *Brucella* Biovar 4 was cultured.
  - Herd is under quarantine with a herd management plan in place.
  - Three negative whole herd tests will be required for release of quarantine.
- A small beef herd was identified as a result of an MCI trace. A whole herd test found five reactors and one suspect.
  - Animals were slaughtered or spayed and culture results identified *Brucella* Biovar 1.
  - Herd is under quarantine and a herd management plan is in place.
  - Three negative whole herd tests will be required for quarantine release.

USDA-APHIS-Veterinary Services conducted a review of Idaho's brucellosis program in September 2012. The review team identified the following strengths and recommendations relative to Idaho's program:

Strengths:

- Good utilization of individual herd management plans for producers within or using Idaho's DSA.
- Mandatory testing required for cattle herds with known elk/cattle interaction.
- Rules prohibiting the private feeding of big game animals.

Recommendations:

- Expansion of Idaho's DSA to include area around recently identified positive cattle herd.
- Work with the Idaho Department of Fish and Game to enhance wildlife surveillance in areas around Idaho's DSA.
- Enhance enforcement of movement testing requirements for cattle leaving the DSA.
- Enhance public outreach regarding brucellosis risk mitigation.

Wyoming Report Summary

Dr. Jim Logan
Wyoming State Veterinarian

Wyoming currently has one herd of domestic bison under quarantine for Brucellosis. This herd was initially placed under quarantine after finding two positive animals in a routine, change of ownership test in the fall of 2010. All suspect/positive animals have either been sold for slaughter or are under strict isolation and are spatially separated from the rest of the herd until they can be fed and conditioned for slaughter. This herd is within the boundaries of Wyoming's Designated Surveillance Area (DSA).

Wyoming recently released the quarantine on an affected cattle herd which was identified in the fall of 2011. This herd was found on routine change of ownership testing and released following three negative herd tests and post calving testing. An assurance test will be done in the fall of 2012.

Wyoming requires calfhood vaccination statewide, and all sexually intact female cattle that inhabit the DSA must be calfhood or adult vaccinated. From July 1, 2011 to June 30, 2012, 198,572 head of cattle were vaccinated – this includes calfhood, adult and yearling booster vaccinations. There are 52 herds conducting adult and/or yearling booster vaccinations, which account for 9,581 of the total head vaccinated statewide. The Wyoming Livestock Board (WLSB) also has a statewide identification requirement whereby all sexually intact female cattle 12 months of age and over must be officially identified prior to any change of ownership. Additionally, all sexually intact female cattle regardless of age that are in the DSA at any time must be officially identified prior to moving from the DSA.

All female cattle from the Wyoming DSA sold for breeding purposes (regardless of age) and all females over 18 months of age are required to be tested within 30 days prior to change of ownership, movement from the DSA, and interstate movement. Between July1, 2011 and June 30, 2012, 36,023 animals were tested. Of that number, 110 tested positive (all but three of these were from the previously mentioned bison herd), and six were suspects. We expect to find occasional cases of Brucellosis among our cattle herds as long as there is a wildlife reservoir of the disease in our state. Our test and identification requirements provide good surveillance, traceability, and early detection. The WLSB Brucellosis requirements are well enforced through brand inspection since any change of ownership or inter-county and interstate movements must include a brand inspection clearance. There are currently 272 herds located or grazing in the DSA with partial or whole herd tests done. The total state cost for surveillance testing and vaccination is $218,989.50.

There are 432 producers in the DSA, of which, 162 have herd plans. This equates to approximately 37.5% of DSA producers.

Wyoming, along with Idaho and Montana, underwent an APHIS review in September, 2012. Recommendations for Wyoming's Brucellosis management/prevention/surveillance were: 1) Continue slaughter surveillance; 2) Assure commuter herd compliance; 3) Increase the number of herd plans; and 4) Lowering test-eligibility age. Commendations from the review team were: 1) Buffer zone built into the DSA; 2) Brand Inspection system enhances compliance; 3) Laboratory capacity and function; and 4) Wildlife agency surveillance, risk mitigation, and cooperation with animal health officials is good.

Consortium for the Advancement of Brucellosis Science

Dr. Walt Cook

University of Wyoming

To further the goal of promoting brucellosis vaccine and diagnostic research, the state of Wyoming has provided seed funding for a scientific approach, the Consortium for the Advancement of Brucellosis Science (CABS). This consortium consists of stakeholders and scientists from around the country who have identified gaps in current research, secured some funding and conducted outreach for the advancement of brucellosis science worldwide. For full vaccine and diagnostic test development, CABS or some other entity must receive large-scale funding so complete research on those candidates can be conducted.

The CABS Scientists' Meeting occurred on June 14, 2012 at the Horse Barn Theater, Wyoming Territorial Prison in Laramie, Wyoming. Presentations were heard from:

- Brant Schumaker from the University of Wyoming (UW) who discussed RB51 Safety Studies. This study had three groups:
    1. Calfhood vaccinates without any subsequent vaccination (Controls);
    2. Calfhood vaccinates with adult vaccination (AV) while pregnant (AV); and
    3. Calfhood vaccinates with booster vaccination (BV) and AV while pregnant (BV+AV).

Animals were bred in the Fall of 2011 and monitored for signs of reproductive loss. Preliminary results indicate that losses were minimal.

- Jeff Adamovicz, also from UW discussed RB51 Immunology Studies. His team is looking at CMI (T cells) to determine if cattle boosted with multiple doses of RB51 have a greater T-cell response. They are also attempting to work with Steve Olsen to challenge these cattle in Ames.
- Gerry Andrews of UW explained his Subunit vaccine and diagnostic studies. They are using outer proteins of *B. abortus* for potential vaccines and a lateral flow device for diagnostics. He has done rodent studies for different subunit vaccines – several look promising. They are hoping to start goat trials soon. The lateral flow device is feasible, but still needs work optimizing it.

- Don Davis discussed Texas A&M University (TAMU) studies. There is not much work being done on *B. abortus* at TAMU. Allison Fitch has a microencapsulation laboratory that can do some vaccine work. Oral delivery seems to offer better protection especially if an abrasive is added.
- Dr. Davis also discussed the role genetics plays in susceptibility to *Brucella*. Some bulls are naturally resistant to infection as are their offspring. This may offer producers another mechanism to prevent infection in their herds.
- Walt Cook led a *B. suis* in cattle discussion. In the southern United States and other countries, this is a big problem. Cattle are a dead-end host for *B. suis*, but infection causes major diagnostic (and thus regulatory) problems.
- Nathan Sriranganathan of Virginia-Maryland presented on Chronic Brucellosis: *Immunomodulation and B. suis: Immunocontraception*. Schurig and Sriranganathan and others are evaluating a strain of RB51 that over-expresses SOD and WBOA to see if it increases protection. Results are promising. They are also looking at cross-protection against *B. suis, B. meletensis*, and swine influenza with RB51 leuB and pN54; preliminary results indicate some cross protection against other *Brucella*, not against flu. They are also using a *B, .suis* vaccine with over-expression of GnRH vaccine as immunocontraceptive and *Brucella* vaccine for swine; the results are quite promising.
- Jack Rhyan of APHIS discussed GonaCon and other studies. He discussed the Yellowstone National Park (YNP) bison quarantine project that was able to successfully eliminate *B. abortus* from these bison. Bison went to Ted Turner and Indian reservations. GonaCon (GnRH vaccine) has been shown to be effective in bison. The idea is to vaccinate *Brucella* positive bison so they don't calve/abort and thus do not shed.
- Valerie Ragan of the Virginia-Maryland Regional College of Veterinary Medicine Center for Public and Corporate Veterinary Medicine discussed international issues. Internationally *B. abortusi* is a major human health problem. Many countries would be willing to collaborate in conducting research. She believes with international data on candidate vaccines, we should be able to get a conditional license. The Center for Veterinary Biologics requires safety and reasonable assumption of efficacy of vaccines for such a license.
- Steve Olsen gave an Agriculture Research Services (ARS) research update. He believes we still have a chance at getting *B. abortus* off the select agent list. It will be up for review in the next year. He has found that repeated vaccination with RB51 in bison (eg. 4 doses in a year) does not improve protection. He also discussed ARS facilities; they have modest capacity, but a long waiting list to use them.
- Todd Cornish of UW mentioned the Wildlife/Livestock Disease Center. This will be a great place for collaboration.
- Brandon Scurlock of Wyoming Game and Fish Department (WGFD) reviewed Strain 19 in elk studies. The bottom line: after decades of Strain 19 vaccination on the feedgrounds, there are no data to indicate that doing so is reducing prevalence.
- Phil Elzer discussed research at Louisiana State University (LSU). Select agent issues got so bad that they destroyed their entire inventory of *Brucella*. Their facility has been decommissioned so they can no longer do challenge studies. They use Strain 19 as a model (for field strain infection) in goats. They are also doing work on a human vaccine.
- Jim Logan (Wyoming State Veterinarian) and Phil Elzer then led a discussion on Latent Heifer Syndrome and Don Evans joined by phone. There is concern that heifers exposed in utero may test negative until the time of calving/abortion and thus expose other animals. Now that herds are no longer being depopulated, this is a hypothetical way that brucellosis may get out of the Greater Yellowstone Area (GYA). Many state veterinarians are quite concerned about this.

The following day the stakeholders group met to review scientific progress and discuss ways to find increased funding for *Brucella* vaccine and diagnostic research.

The US Senate version of the Farm Bill (S. 3240) Title XII – Subtitle B – Section 12101 "Wildlife Reservoir Zoonotic Disease Initiative" provides for funding of vaccine and diagnostic tests for Brucellosis, Tuberculosis and other zoonotic diseases with significant wildlife reservoirs. While the Bill would not guarantee funding for CABs, it would allow CABS to compete for funds for which it has been ineligible to compete in the past. When the two versions of the Farm Bill go to conference committee, we may ask for support of the Senate Version.

Mexico Brucellosis Update
Dr. Jose Alfredo Gutierrez
SAGARPA, Mexico

This report in its entirety is included at the end of this report.

ARS Brucellosis Research Update and Select Agent Information
Dr. Steve Olsen
ARS

This report in its entirety is included at the end of this report.

Brucella Diagnositc Research – Lipidomics of Various _Brucella_ and Yersinia

Dr. Torsten Eckstein
Eckstein Diagnostics, Inc.

Brucellosis is a zoonotic infection transmitted from animals to humans caused by *Brucella* ssp including *B. abortus*, *B. suis*, and *B. melitensis*. Brucellosis is a serious livestock disease that has significant animal health, public health, and national and international trade consequences. Unfortunately, current diagnostics detect mostly false-positive animals and reduce the speed of success for the ultimate goal of the national brucellosis program to establish a national disease-free designation.

The mostly trusted diagnostic test for brucellosis is the fluorescence polarization assay (FPA), although several other tests provide good to excellent sensitivity. The unacceptable low specificity is probably due the cross-reactivity with *Yersinia* infection in tested bison. Although most tests identify all brucellosis animals, from an eradication-standpoint, false-positive animals do not reduce the efficiency of the eradication process. It definitely affects bison and elk farms and the reason for high costs for the eradication program.

Recently, the detection focus moved partly to dairy products derived from unpasteurized milk that seemed to be imported to the US. The key evidence for dairy products containing live *Brucella* ssp. is the cultural identification of this pathogen. However, due to the classification as a group B select agent, the cultural identification of *Brucella* ssp. is restricted to few, BSL-3 level approved laboratories and thus, additional detection methods are necessary for screening dairy samples without growing the pathogen. The detection of parts of the pathogen seems to be the most reliable detection method and among those molecules lipids are the most promising pathogen-specific molecules.

We have identified eight *Brucella*-specific lipids that have the capability to serve as diagnostic tools. At least of these lipids was further structurally characterized and identified as ornithine lipids. We were able to synthesize a similar lipid that has excellent reactivities in serological ELISAs with sera from infected cattle. We also demonstrated that the lipid profiles of *Brucella* ssp. could be used to differentiate between Brucella from marine mammals and *B. abortus*, *B. suis*, and *B. melitensis*. Finally, we demonstrated that the *Brucella*-specific lipids could be used to detect the pathogen in milk and/or dairy products.

The Role of Host Genetics in Differential Susceptibility to _Brucella abortus_: A Bovine Model

Dr. Chris Seabury
Texas A&M University

Differential susceptibility to brucellosis in domestic cattle is known to be genetically controlled. A total of 66 historic DNA samples representing cattle that were previously experimentally challenged with 10^7 CFU of live *Brucella abortus* strain 2308 (conjunctival administration) and also evaluated using *in vitro* macrophage challenge assays were available at Texas A&M University for a genome wide association study (GWAS) that employed the new Illumina BovineHD Assay (777K). Nearly all of the genotyped cattle were Angus crossbreds derived from 15 sires and 40 dams. The classification of cattle into “resistant” (n = 32) and “susceptible” (n = 34) phenotypes was based on post-challenge serological titers, abortions, bacteriologic cultures of 50 unique tissues harvested at slaughter, and/or *in vitro* macrophage challenge assays. For the *in vivo* experimental challenge, the “susceptible” phenotype was defined as any cow or bull for which *B. abortus* was cultured from at least one investigated tissue, whereas the “resistant” phenotype was only assigned to those cattle for which zero *B. abortus* were cultured from all investigated tissues. This phenotyping strategy essentially created a binary trait classification scheme that included one extreme category (i.e., resistance), because recovery of even a single *B. abortus* colony from one tissue was interpreted as an indication of susceptibility. Importantly, even though susceptible cattle display a spectrum of bacteriological phenotypes ranging from one colony forming units (CFU) derived from a single tissue, to many CFU recovered from multiple tissues, the resistant cattle represent a uniform and extreme disease phenotype. Therefore, because resistance to brucellosis in domestic cattle is under genetic control (*in vivo* and *in vitro* challenges), with resistant cattle representing a true phenotypic extreme, we hypothesized that the disease classification strategy would essentially create significant disparities in the distributions of alleles and genotypes for loci modulating resistance, thus enabling detection of those loci with very few samples. To test this hypothesis, we used several inheritance models in conjunction with logistic regression and the correlation trend test with principal component (PC1 & PC2) correction for stratification. Collectively, using 66 samples, we detected at least 5 potential autosomal signatures of association that surpass the minimal unadjusted *P*-value for moderate evidence of association (5×10^{-5}), as recommended by the Wellcome Trust Case Control Consortium. No associations were detected on BTAX.

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Bovine Brucellosis Latency/Latent Heifer Syndrome

Dr. Don Evans

USDA-APHIS-VS

This is a review of literature on the issue of latent brucellosis infections and diagnostic testing in cattle. This presentation also includes some insight and information from years of managing brucellosis affected herds as a Designated Brucellosis Epidemiologist.

Dictionary definitions:

Latent period – "The incubation period of an infectious disease."

Recrudescent – "To break out again after a dormant or inactive period."

There are some good literature reviews on the issue of latent carrier cattle written by Ray in 1977, Nicoletti in 1980, Sutherland in 1980 and Sutherland and Searson in 1990.

The latent period is directly related to *B. abortus'* ability to survive for prolonged periods in relatively low numbers within the host. After being phagocytized by macrophages, the bacteria are transported to tissues in the lymphoid system where in some animals, they are able to survive or evade the host's immune system. The pathogen's ability to persist undetected by the host immune system results in latent carriers. The occurrence of latent carriers among cattle (heifer syndrome) is widely accepted (Plommet et al. 1973, Lapraik et al. 1975, Crawford, et al 1986), Latency associated with *B. abortus* is problematic for disease management because infected reproductively immature animals can test negative on serologic tests but shed *B. abortus* when reproductively mature. One experimental cattle study found approximately 18% (4 of 22) of calves born to experimentally infected mothers, were latently infected (Plommet, et al. 1973). These heifers were separated from their dams at birth without nursing; bottle fed separately until weaning; and then after breeding, kept in isolation until calving or abortion (Lapraik et al. 1975). A thorough epidemiological study conducted by Wilesmith (1978) estimated that 2.5% of heifer calves born to serologically positive dams reacted in early adulthood and constituted a risk to newly established herds. In a group of thirty-seven heifers from serological reactors in three Texas affected herds, two heifers (5.4%) were found to be shedding *B. abortus* at parturition – (Crawford, et al 1986). One heifer in this study was not serologically positive until two weeks prior to parturition, but the other was serologically positive on the first post-weaning test. Two additional studies found none of fifty-one and none of ninety-five heifers born to serologic reactors in affected herds to be culture or serologically positive at parturition (Dolan, L., 1980, Ray, et al, 1988). There is one report (Lapraik and Moffat, 1982) in the literature where *Brucella* shedding and seroconversion was delayed for nine years following calfhood exposure. Nagy and Hignett (1967) fed heifer calves large numbers of *Brucella abortus* every day for the first 15 days of life and re-exposed them at 7 months. They concluded that the neonatal infection led to a degree of immunity against subsequent exposure. Two of four calves not exposed until 7 months of age, became permanently infected.

The incubation period for bovine brucellosis is highly variable ranging from ten days to years – typically one to seven months. Some of the major variables influencing the incubation period include previous exposure or vaccination and genetic resistance which tend to lengthen the incubation period. Incubation will vary by exposure dose, virulence of challenge strain and stage of gestation at exposure. Prepubescent heifers and male cattle seem to be more resistant, thus will likely have longer incubation periods.

During a study of 181 brucellosis affected herds in Louisiana from 1989 to 1992, based upon assessment by field veterinarians, the sources of infection to the herds were found to be: purchased additions in 43% of the herds; recrudescence or latent infection in 31% of these herds; adjacent or contact exposure in 22% of the herds; undetermined in the remaining 4% of the herds. During this time period, vaccinated heifers were allowed to leave affected herds without restriction and quarantines could be released after testing negative six months following the removal of reactors. Many of the herds with a purchased source of brucellosis were found to have acquired brucellosis by purchase of heifers that originated from herds under quarantine.

Dr. Don Cheatham, former USDA-APHIS-VS Area Epidemiology Officer in Alabama (retired), presented data in 1993 where 25% of newly infected herds in Alabama and Tennessee were the result of recrudescence or seropositive animals identified after quarantine release that had exposure during previous herd quarantine.

These variables in incubation period and the acknowledgement of latent brucellosis infections, lead to the recommendations in the 1997 Brucellosis Emergency Action Plan to put an emphasis on depopulation of affected herds. It was recommended that herds not depopulated be maintained under quarantine until having all negative tests over a twelve month testing period. It was further recommended that heifers should not be retained except under dire circumstances.

Attempts to be able to determine which animals have been latently infected have been ongoing for years.

Tacken (1964) suggested that vaccination with Strain 19 or a killed adjuvant vaccine would produce stronger and longer lasting antibody levels on the Complement Fixation test in infected heifers. Using this technique he identified 35 heifers out of 554 head on 75 farms but did not provide further data to support this hypothesis.

Anamnestic responses following vaccination with a killed Strain 45/20 vaccine were evaluated by several investigators as a technique to detect latent infections. (Cunningham, 1968; Cunningham and O'Connor, 1971; Reid and Harvey, 1972; and Nicoletti, 1977) - Conclusions from these studies are that the Coombs and Complement Fixation tests can be used post vaccination with killed Strain 45/20 to identify an anamnestic response and in one study they were able to isolate *Brucella* from 24% of the Complement Fixation positives and 19% of the Coombs test positive animals. Nicoletti (1977)

found in 5 herds studied for an anamnestic response, that no further infected animals were found in one herd upon further testing, but the other four herds had a significant percent of animals seroconvert afterwards. Strain 19 vaccinates were found to have a similar anamnestic response as infected animals, which complicates the use of the anamnestic test in vaccinates, Strain 19 vaccinates will exhibit an antibody response to the Strain 45/20 vaccination.

Weynants, et al (1995) conducted a study that demonstrates the difficulties in detecting exposed animals with multiple testing techniques. They challenged ten animals (six nonpregnant and four in the first weeks of pregnancy) with 6×10^7 viable *B. abortus* strain 544. The ten challenged animals were necropsied on day 80 with selected tissues cultured. The complement fixation and serum agglutination tests were positive for three animals at day 25 with only one positive by day 77. The Rose Bengal test found two positive by day 20 and detected nine out of ten by day 35, but only one was positive by day 77. The ELISA test performed probably the best by detecting ten out of ten for days 45 through 60 and nine out of ten by day 77. The gamma interferon test detected seven out of ten from days 25 through day 55 and was positive for all ten animals by day 77. When compared with culture results on day 77 the standard serologic tests only detected one of six infected animals, ELISA, DTH (Brucellin) and gamma interferon were positive on all culture positive animals. The ELISA was positive for three of four culture negative animals, the DTH was positive for two out of four and the gamma interferon was positive for four of four culture negative animals. None of the tests identified all of the exposed and potentially latent animals throughout the study period. The authors noted that the gamma interferon test would probably not be able to distinguish Strain 19 vaccinates from infected animals.

In summary, latent brucellosis infections have been recognized for years at rates of from zero to eighteen percent. Multiple variables are involved that determine the likelihood of an animal being latently infected or incubating the disease. No one test has been found that can detect all animals with a latent infection. Vaccination may interfere with an accurate diagnosis with some of our current tests and tests under development, although I was unable to find any studies exploring how Strain RB51 vaccinates would respond to the above mentioned tests for latent infections. Until a test is found that can detect a high percent of vaccinated animals that have been exposed to an infective challenge dose of *Brucella*, or a vaccine is developed that is efficacious in preventing infection, exposed animals should be restricted until current testing provides a high probability that the animal is not latently infected. This means intact heifer animals should at least be restricted until they have had a negative post-calving test.

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Committee Business:

Two resolutions were brought before the committee for discussion:

1) Use of RFID in Brucellosis Vaccinated Cattle; Waiver of Tattoo Requirement; Consistency in Ear Placement; Continued Funding of Ear Tags.

This resolution was tabled until next year's meeting.

2) Brucellosis in the Greater Yellowstone Area.

This resolution was passed and forwarded to the Committee on Nominations and Resolutions.

REPORT OF THE SCIENTIFIC ADVISORY SUBCOMMITTEE ON BRUCELLOSIS

Chair: Walt Cook, WY

Introduction of sub-committee members.

Members present: Don Evans, KS; Steve Olsen, IA; Val Ragan, MD; and Walt Cook, WY.
Members absent: Jack Rhyan, CO; Don Davis, TX; and Phil Elzer, LA.
Numerous visitors from various countries, industry, federal, state, etc. attended the joint meeting.

Review of Data for the Ability of Western Blot to Discriminate *Yersinia* from *Brucella* in Cervids

Neil Anderson led this discussion based on data previously provided. In addition, Terry Kreeger discussed a controlled experiment conducted by the Wyoming Game and Fish Department. Steve Olsen mentioned that in the controlled experiment when elk were initially exposed to Strain 19 and subsequently exposed to *Yersinia*, they exhibited an anamnestic response; this did not occur when exposed to *Yersinia* followed by Strain 19.

After reviewing the data, the Subcommittee agreed that there is no way to reliably discriminate *Brucella* vs. *Yersinia* infection using currently available serologic tests. When serologic titers to brucellosis are found in areas where exposure is not expected, we recommend an epidemiologic investigation be used to determine actual status. This may require additional sampling.

Review of response to last year's resolutions

#24: Use of Buffered Acid Plate Antigen (BAPA) and Flourescent Polarization Assay (FPA) in Cervids and # 26: Calfhood Vaccination of Bison up to 24 months of Age). Both of the responses to these resolutions were positive. APHIS-VS agreed to incorporate the BAPA and FPA as official tests. However, APHIS-VS believes that safety and efficacy of RB51 vaccine in bison up to 24 months of age needs to be evaluated. They requested the Subcommittee to evaluate relevant data.

There are currently little controlled experimental data on the efficacy of RB51 in older bison. However, Steve Olsen is conducting such an experiment which will be completed in 2014. The subcommittee agreed to evaluate that data when it is available.

Council of State and Territorial Epidemiologists (CSTE) recommendation to develop a *B. canis* test for humans. The Council of State and Territorial Epidemiologists is recommending that the Centers for Disease Control and Prevention, the National Institutes of Health, and Food and Drug Administration aid in the development of a reliable assay to detect *B. canis* antibodies in human serum and that data generated from the use of such a test be shared with state health departments and departments of agriculture. CSTE is asking for our support of this recommendation.

The subcommittee urges the Brucellosis Committee to support this recommendation for several reasons:

1. It fits the notion of "One Health",
2. Public health funds will be used and should not detract from funds used for veterinary *Brucella* work,
3. *B. canis* is a rough strain and we need better serologic tests to distinguish rough from smooth strains,
4. Antigens used for *B. canis* serology in animals are no longer being produced. If a human assay is developed, perhaps antigens could be shared.

In addition, the subcommittee recommends that when humans are found to have titers to brucellosis, an effort will be made to determine the species. We further recommend that anytime a human is determined to be infected with brucellosis that information should be shared with the veterinary side and an epidemiologic investigation conducted to determine the animal source.

Bovine adult vaccination-induced titers to CF/FPA (continuation of last year's discussion)

There is concern expressed by some producers in the Greater Yellowstone Area that adult and booster vaccination with RB51 may subsequently result in cattle with titers on serologic tests. The subcommittee agreed that such an occurrence is a very rare event as RB51 lacks the O-side chain that would cause such titers. We feel that it is important for producers to recognize that occasional titers on screening tests is to be expected whether cattle are vaccinated or not. This is why we rely on confirmatory tests and, if necessary additional follow-up. There are an array of factors which may cause nonspecific reactions on serologic tests (including exposure to Yersinia and other non-Brucella organisms). The probability of adult/booster vaccination causing titers is very low.

Application of novel antigenic proteins of *Brucella abortus* as diagnostic targets and sub-unit vaccines

Gerry Andrews of the University of Wyoming gave a presentation to the subcommittee and guests on his work examining the above.

REPORT OF THE FERAL SWINE SUBCOMMITTEE ON BRUCELLOSIS AND PSEUDORABIES

Chair: Joseph Corn

Dr. Joseph Corn, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, provided an update on the National Feral Swine Mapping System (NFSMS). SCWDS began producing nationwide feral swine distribution maps in 1982 by working directly with state and territorial natural resources agency personnel. In 1982, 17 states reported feral swine in a total of 475 counties. With support from USDA-APHIS-Veterinary Services (VS) the SCWDS developed and implemented the National Feral Swine Mapping System (NFSMS) in 2008. The NFSMS is an interactive data collection system used to collect and display current data on the distribution of feral swine in the United States. The feral swine distribution maps are produced using data collected from state and territorial natural resources agencies, USDA-APHIS-Wildlife Services (WS), and other state/federal wildlife and agriculture agencies. The 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 distribution map is updated with verified additions on a monthly basis. Feral swine populations and/or sightings are designated either as established 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. Over 500 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/. Additional data are provided to state/federal agencies and universities on request. Although the distribution of feral swine continues to increase in the United States, feral swine were recently eradicated from Nebraska. Established feral swine populations were reported in 37 states in 2011, but currently in 2012 are reported as present in 36 states.

Dr. Troy Bigelow, USDA-APHIS, Veterinary Services (VS), National Center for Animal Health Programs (NCAHP), gave a presentation on USDA, APHIS swine health activities including activities in Pseudorabies, Swine Brucellosis, Classical swine fever (CSF), Swine Health Protection Act, Trichinae and Flu Surveillance. Major items discussed included the Swine Brucellosis and PRV concept paper. The paper is drafted and is proceeding through the clearance process prior to publication. USDA explained that FY 2012 indemnity funds were used to purchase three pseudorabies virus (PRV) infected herds and three herds for swine brucellosis. Additionally, USDA is currently drafting a proposed interim rule to modify the definition of swine brucellosis validated free state to allow flexibility and surveillance options. All surveillance samples tested in FY 2012 were negative for CSF. Swine Health Protection Inspection activities continued in FY 2012. A total of 125 non-licensed feeders were found out of 36,366 searches performed. USDA collaborated with other regulatory agencies in Flu Surveillance. In FY 2012 nearly 300 people have documented illness traced to exposure to swine events. Events include fairs and exhibitions. Most cases occurred in pig exhibitors and have occurred after close contact to swine. USDA continues to participate in flu surveillance activities. These activities allow the USDA to identify the common flu viruses circulating in the swine population. The USDA also continues to monitor disease occurring in other regions of the world including monitoring the occurrence of African swine fever (ASF).

Dr. Tom Ray, North Carolina Department of Agriculture and Consumer Services gave a presentation on feral swine control efforts in North Carolina. The 2009 General Assembly passed an Act to create a Feral Swine Study Committee to Direct the Department of Agriculture and Consumer Services to study issues related to the importation of feral swine in North Carolina, including associated risks, economic impact, population estimates, disease risks, enforcement issues and penalties for the illegal transportation of feral swine into and around the state. Background was provided relative to the importance of agriculture in general to the state and the swine industry in particular, and on the economic impact of introduction of a foreign animal disease (FAD) into commercial swine resulting from interactions with feral hogs. Control measures that the Study Committee considered and discussed were presented along with the final recommendations that came out of this Committee. The resulting legislation aimed at controlling feral swine in the state was provided in detail along with early results from those efforts.

Dr. Tom Gidlewski, USDA, Wildlife Services (WS), National Wildlife Disease Program (NWDP) gave an update on FY 2012 monitoring of feral swine diseases. In FY 2012, serum samples were collected from 2891 animals in 29 states. Diseases monitored were CSF, PRV, Swine Brucellosis, SIV, Hepatitis E, Trichinellosis, Toxoplasmosis, and Leptospirosis for part of the year. CSF testing was done at Foreign Animal Disease Diagnostic Laboratiory (FADDL) and the Texas A&M Veterinary Diagnostic Laboratory. No CSF exposure has been found. PRV testing was done at two NAHLN laboratories, using the gB ELISA test. Prevalence appears to be about 18%. Swine brucellosis testing was done at the Kansas State-Federal Laboratory until July, 2012, when the Kentucky Brucellosis Laboratory took over all brucellosis testing. All samples are tested by the Fluorescence Polarization Assay (FPA). Apparent prevalence is 6-7%. Over the past two years, over 200 tonsils from swine in eight states have been cultured for brucellosis. Of these, 21 have been culture positive, and all *Brucella suis*. SIV testing is done in-house using ELISA. Prevalence is about 7 %. Matching nasal swab samples from sero-positive swine are tested by rtPCR at the NVSL. *Trichinella* and *Toxoplasma* testing has been done by the ARS in Beltsville, MD, most recently on FY 2011 samples. No FY 2012 samples have been tested yet. Prevalence of *Toxoplasma* is about 15%, while *Trichinella* exposure is about 2%. Tongues of swine from counties with high prevalence of seropositive animals, are tested for *Trichinella* larvae, and *Toxoplasma tachyzoites*. For leptospirosis, serum and kidney samples were collected for part of the year, in a study that will continue into FY 2013.

Diagnostic testing is being done at Colorado State University. Results are pending. In a retrospective study, serum samples were sent to the North Carolina NAHLN laboratory (Rollins) to screen for porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus type 2 (PCV2). Both were present in feral swine, with PCV 2 more common than PRRS. But feral swine appear to be spillover hosts that don't sustain PRRS, and probably not PCV2.

REPORT OF THE SUBCOMMITTEE ON GREATER YELLOWSTONE AREA (GYA)

Chair: Marty Zaluski, MT

The Subcommittee met on October 21, 2012 with Chair, Marty Zaluski calling the meeting to order at 12:30 p.m. The subcommittee meeting was held in conjunction with the Scientific Advisory Subcommittee and the Feral Swine Brucellosis and Pseudorabies Subcommittee.

Subcommittee members present included: Terry Kreeger, Jim Logan, Dave Hunter, Bill Barton, Michael Gilsdorf, Neil Anderson and Marty Zaluski. Subcommittee members absent included: Chuck Massengill, Rick Wallen, and John Belfrage, and Mark Drew. Susan Keller attended part of the session.

Neil Anderson, Montana Department of Fish, Wildlife and Parks (FWP) presented on factors affecting elk group size and impacts on brucellosis seroprevalence in Montana's GYA. Spatial variations in elk density and aggregation patterns across the Montana portion of the GYE (Greater Yellowstone Ecosystem) were used to generate predictions of elk to elk disease transmission risk. These predictions were validated using current estimates of brucellosis seroprevalence. Snowpack, vegetative cover type, and elk densities affected elk group sizes and percent grasslands within the winter range and elk density affected the proportion of the population aggregated in large groups (>300 elk). Increasing elk herd density not only increased predicted average group size and proportion of the population aggregated in large groups, but increasing elk density also strongly increased the size of the largest elk aggregations. The study found no evidence that wolf predation risk, measured as an annual wolf:elk ratio, affected mean group size or the proportion of the population aggregated in large groups.

Brant A. Schumaker presented on a study that evaluated three RB51 vaccination regimens based of their reproductive effects on cattle. A herd of cattle (N=616) was randomly divided into three treatments groups: 1) Calfhood vaccinates without any subsequent vaccination (Controls); 2) Calfhood vaccinates with AV while pregnant ; and 3) Calfhood vaccinates with a booster vaccination as non-pregnant yearlings, followed by AV while pregnant (BV+AV). Pregnancy losses were estimated as the difference in the number of pregnant animals during Fall pregnancy checks and the number of previously pregnant cattle without live calves one week after parturition in the Spring. All animals were treated as a single population with no differences in management. The overall pregnancy success of the herd was high (98.4%) with only ten calves lost. Due to the management practices of the herd, tissue samples from only two of ten live-born calf carcasses were available for analysis. Test results from these specimens did not indicate RB51 or an infectious cause for death. No meaningful or statistically significant differences were seen between treatment and control groups (Control: 3/207 lost; AV: 4/204; BV+AV: 3/205). We conclude that adult vaccination with RB51 did not result in detectable decreases in the proportion of calves born live.

Bill Barton, Jim Logan, and Marty Zaluski presented on the recently completed Brucellosis Management Area review of Idaho, Wyoming, and Montana by USDA. The report was not finalized prior to the USAHA, but a draft was made to the three states for review.

The meeting adjourned at approximately 2:30 p.m.