REPORT OF THE COMMITTEE ON BRUCELLOSIS

Chair: Glenn Plumb, Yellowstone National Park, WY
Vice Chair: Claude E. Barton, Nashville, TN

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The Committee met on Wednesday, October 18, 2006, from 8:00 a.m. to 12:30 p.m. at the Minneapolis Hilton Hotel, Minneapolis, Minnesota during the 110th Annual Meeting of the United States Animal Health Association (USAHA). A total of 124 individuals were in attendance of which 59 were members and 65 were guests. The meeting was Chaired by Glenn Plumb and there were 16 scientific presentations, reports, resolutions, and recommendations presented to the Committee for consideration.

Dr. Claude Barton gave a brief review of the 2005 meeting, resolutions, and recommendations. Two resolutions and four recommendations had been forwarded to the Secretaries of the United States Department of Agriculture (USDA) and the United States Department of Interior (USDI). There were responses to all six documents, all of which were considered favorable.

Drs. Debra Donch and Arnold Gertonson, Veterinary Services (VS), Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), presented the FY 2006 Annual Status Report of the Cooperative Brucellosis Eradication pProgram. At the beginning of the year there were three states classified Class A for brucellosis; Idaho, Texas and

Wyoming. Wyoming regained Class Free status during the year. Idaho and Texas are currently in the final stages of qualifying for Class Free status. A total of two new brucellosis affected herds were disclosed during FY 2005 with both being in Idaho, a state that had been classified as brucellosis Class Free since 1991. Both of these herds were located in eastern Idaho and were depopulated with indemnity. The most probable source of brucellosis in these two herds was infected elk that migrate through the area. Dr. Gertonson reported on the brucellosis activities with wildlife in the Greater Yellowstone Area (GYA) and on the studies and activities in which APHIS-VS is involved. The complete text of the FY 2005 National Brucellosis Status Report is included in these proceedings.

Dr. Eric Ebel, National Surveillance Unit (NSU), VS-APHIS-USDA gave a paper entitled, Planning Cattle Brucellosis Surveillance. Dr. Ebel's presentation dealt with the development of plans for brucellosis surveillance after all 50 states achieve Class Free brucellosis status. The complete text of this presentation is included in these proceedings.

Dr. Bob Hillman, Texas State Veterinarian, gave a brief report on the status and progress of the brucellosis program in Texas. Dr. Hillman reviewed the Texas program history since 1994 when the state achieved Class A status. He detailed some of the frustrations of trying to complete the state's brucellosis program where an extremely low, but persistent level of brucellosis existed in a large cattle population. Currently Texas is one of only two Class A states in the U.S. The last known affected herd was released in September, 2006, and Texas has completed the required 12 month consecutive period without a known brucellosis affected herd. They are conducting additional epidemiologic evaluations in high risk areas before applying for Class Free status. In May of 2006 the Chairman and Executive Director of the Texas Animal Health Commission (TAHC) appointed a Brucellosis Eradication Working Group with the charge to assess and address areas of potential weakness in the Texas brucellosis program to assure the rapid elimination of the last vestiges of cattle brucellosis.

Dr. Phil Mamer, Idaho Fish and Game Department and Mr. John Chatburn, Idaho Department of Agriculture, presented a brief status report of the Idaho brucellosis program. They presented details of the outbreaks in 2005 that resulted in the loss of Class Free brucellosis status along with the epidemiologic evidence linking the outbreak to infected wild elk. By the end of 2006 the time requirement of 12 consecutive months without an affected herd will be completed and Class Free status can be restored provided there are no additional affected herds disclosed in the meantime.

Dr. Dwayne Oldham, Wyoming State Veterinarian gave a status report on the brucellosis program in Wyoming. He reviewed the circumstances of four isolated cases of brucellosis in cattle herds from 2003 to 2005 and the epidemiological evidence linking these cases to infected elk as the most probable source. Wyoming had been brucellosis Class Free from 1985 to 2004 when that status was suspended because of the four cases mentioned above. Following completion of requalification requirements, brucellosis Class Free status was restored to the State of Wyoming on September 15, 2006. Dr. Oldham also

described brucellosis management that is being implemented in Wyoming to prevent additional wildlife-livestock transmission.

Dr. Frank Galey, Dean, College of Agriculture, University of Wyoming and Chair, Wyoming Brucellosis Coordination Team, gave a follow-up report on the activities of the team during 2006. The team presented 28 recommendations in the initial report which were accepted and funded by the Governor and the Wyoming State Legislature. The team met twice in 2006, in May and in September. Major issues addressed by the team included commingling of wild elk and cattle, the State's brucellosis status, efforts to address brucellosis in wildlife and cattle, legislation, litigation regarding the experimental test and removal project in an elk herd unit, and the memorandum of understanding related to the Greater Yellowstone Interagency Brucellosis Committee (GYIBC). The appointment and funding for the team were extended through 2007.

Mr. John Treanor, Yellowstone National Park (YNP), gave a Time Specific Paper entitled, Brucellosis in Yellowstone Bison: An Individual-Based Simulation Model of Vaccination Strategies. The complete text of this paper is included in these proceedings.

Dr. Francisco Roberto, Idaho National Laboratory, presented a Time Specific Paper entitled, Application of a Real-Time PCR Assay for *Brucella abortus* in Wildlife and Cattle. The complete text of this paper is included in these proceedings.

Dr. Mark Atkinson, Montana Department of Fish, Wildlife and Parks, gave a presentation entitled, *Yersinia/Brucella* Cross-Reactivity in Rocky Mountain Elk. The complete text of this presentation is included in these proceedings.

Dr. Torn Linfield, Montana State Veterinarian, presented a report on the Interagency Bison Management Plan for the State of Montana and Yellowstone National Park. Further details are presented in these proceedings.

Dr. Jack Ryan, VS-APHIS-USDA gave an update report on the Bison Quarantine Feasibility Study. Goals of the study are; 1) to investigate the feasibility of using the bison quarantine protocol published in the Uniform Methods and Rules (UM&R), or modified protocol if needed, to result in brucellosis-free bison from the infected Yellowstone herd for use in bison restoration; 2) to investigate the feasibility of quarantine to conserve genetics from the Yellowstone herd by creating additional conservation bison herds that are brucellosis-free in other habitats in North America and; 3) to investigate quarantine as a potential tool for use in managing GYA bison populations. The study was called for in the Record of Decision of the Interagency Bison Management Plan (IBMP) signed in 2000. Environmental assessments were conducted for Phase 1 and Phases 2 and 3. The study began in March 2005 with the acquisition of 17 sero-negative bison calves that were captured in the bison traps. Since the beginning of the study a total of 101 bison have been entered into the project. Of 96 remaining in June 2006, 48 were sent to slaughter where tissues were collected for bacteriologic culture. Carcasses were donated to food banks. Cultures of target tissues were negative for Brucella spp. Culture of other tissues is ongoing. In August 2006, an additional bison from the 2006 group sero-converted, was necropsied, and was culture positive for *B*. *abortus*,

biovar 1. Of the total of 101 bison, six have sero-converted and all six have been culture positive for *B. abortus*, biovar 1.

Dr. J. Lee Alley, Secretary, USAHA, presented the final report from the Special Committee on Brucellosis in the GYA. The Committee was appointed by the USAHA leadership in 2004 and was charged to hold a working symposium to address the research needs for *Brucella* vaccines, vaccine delivery systems, and surveillance diagnostics for elk and bison in the GYA. The symposium was held at the University of Wyoming in August 2005 with 58 expert scientists from the U.S., New Zealand, Russia, and Canada in attendance. The USAHA Laramie Agenda and Technical Report from the Symposium were released in September 2006 and are available on the USAHA website (www.usaha.org/pubs). The Technical Report and the Laramie Agenda identifies research needs, a timeline for completion, and estimated costs. The Laramie Agenda further identifies the need to enhance old partnerships, develop new partnerships, and work together in completing the effort to eliminate brucellosis in the U.S.

REPORT OF THE EDUCATION SUBCOMMITTEE ON BRUCELLOSIS

Although the Subcommittee has been inactive and without a Chair for two years, eight USAHA members showed up at the scheduled meeting at 10:00AM on October 17. Dr. Glenn Plumb convened the group to discuss the future of the Subcommittee. After a lengthy discussion, a motion was made to discontinue the Subcommittee. The motion was duly seconded and passed unanimously by vote of USAHA members present. This motion was validated by unanimous vote of the full Committee on Brucellosis during its Annual Meeting on October 18, 2006.

REPORT OF THE FERAL SWINE SUBCOMMITTEE ON BRUCELLOSIS AND PSEUDORABIES,

CO-CHAIRS: CARTER BLACK
JOE CORN

The Subcommittee was called to order by Carter Black on October 16, 2006 at 1:00 p.m. There were forty attendees with ten subcommittee members present.

Dave Pyburn presented the USDA update. In FY 2006 there were twelve swine herds infected with pseudorabies and thirteen herds infected with brucellosis. Six of these herds had dual infections. Missouri, Iowa, Wisconsin and Pennsylvania initiated educational programs in FY 2006 to make producers aware of the risk from exposure to feral swine.

Ned Hahn reported on Molecular Epidemiology of Pseudorabies in Feral Swine. The goals are to fingerprint pseudorabies DNA from recent outbreaks, to improve the data base of sequences of virus from domestic and feral swine and to determine sites of virus latency, reactivation and transmission. Polymerase chain reaction (PCR) is used to amplify viral DNA from virus isolates or from infected tissues. The amplified DNA segment is sequenced and compared by phylogenetic analysis. Real time PCR gives the ability to quantify how much virus is in tissue and determine sites of latency and reactivation in feral pigs. In

conclusion viral sequences have been completed from several areas where feral swine are present.

The United States is a pseudorabies melting pot. The predominant genotype in the Southeast appears to be moving north. Some strains of virus are derived from domestic pig virus or vaccines but can be distinguished from the feral swine strains.

The APHIS, Wildlife Services, update was presented by Seth Swafford. Wildlife Services partner with federal agencies, state wildlife agencies, state agriculture departments, domestic swine industry, laboratories and wildlife and animal health organizations. Wildlife Services' wildlife disease capacity is cooperative disease management, research, education and emergency response. The swine brucellosis and pseudorabies surveillance in FY 2006 consisted of 1155 samples from thirteen states. The target for FY 2007 is 2000 samples from twenty-one states. Challenges for this year include program expansion in high risk states, the establishment of a coordinated central data repository, continued interest and involvement and the expanding feral swine population.

Joe Corn, Southeastern Cooperative Wildlife Disease Study (SCWDS) gave an update on studies on the distribution of pseudorabies virus (PRV) and *Brucella suis* in feral swine. The studies included developing maps of the distribution of PRV and *B. suis* in feral swine in the United States, continuing surveys for PRV and *B. suis* in feral swine associated with high density domestic swine production in North Carolina, developing maps of the distribution of transitional domestic swine premises in South Carolina and surveys for PRV and *B. suis* in feral swine associated with transitional domestic swine premises in South Carolina. In 1982 feral swine were present in 475 counties in seventeen states. In 2004 feral swine were present in 1014 counties in twenty-eight states. Factors associated with spread of feral swine are natural dispersal from existing populations, localized escape of domestic swine, localized release of domestic swine, transport and release of feral swine, new populations in areas distant to other feral populations are due to escape or release of domestic and/or feral swine. The database of distribution of *B. suis* in feral swine from 1955-2006 includes published and unpublished data, the records from 16,530 feral swine tested from 194 counties in 18 states and positive records from 1,472 feral swine from 60 counties in 10 states. The database of distribution of pseudorabies in feral swine from 1955-2006 includes published and unpublished data, records from 15,846 feral swine tested from 183 counties in 18 states, positive records from 4,326 feral swine from 103 counties in 10 states. Feral swine and high density domestic swine production maps depict where feral swine and high density domestic production overlap and are used to target surveillance.

Ed Stephens, owner of Two Rivers Outdoor Club, Inc. made a presentation on the U.S. wild boar market. The market for wild boars is the specialty meat market, hunting stock and breeding stock. The Two Rivers Outdoor Club has established a Validated and Qualified swine herd. He encourages USDA and state agriculture agencies to recognize and work with the wild boar industry.

Greg Hawkins, TAHC made a presentation on a state perspective on feral swine. Texas has feral swine in nearly every county. All newly infected transitional swine herds in FY 2006 were epidemiologically linked. In FY 2006 there was disclosure of six cows and one horse infected with *B. suis*. Texas has identified several problems that hamper their efforts to eradicate swine brucellosis in affected herds. These problems include delay in approval of depopulation indemnity, no indemnity for exposed animals and no disposal or transportation funds. Feral swine movements continue to be a problem.

REPORT OF THE SCIENTIFIC ADVISORY SUBCOMMITTEE ON BRUCELLOSIS

CHAIR: PHILIP H. ELZER

The Subcommittee met and was called to order by Phillip Elzer on Oct 17 at 4:00 p.m., with six Subcommittee members and 30 guests present. Subcommittee members present included: Dr. Don Davis, Ms. Barb Martin, Dr. Steve Olsen, Dr. Jack Rhyan. Dr. Davis held Dr. Schurig's proxy and Ms. Martin held Dr. Evans' proxy.

Agenda:

1. Introduction of Subcommittee members.

2. Presentations
    a. Tom Ficht, Texas A&M, presented information on Improved Wildlife Vaccines Through Controlled Release.
    b. Ryan Clark, USDA, presented an update on the fluorescence polarization assay/buffer acidified plate antigen (FPA/BAPA) elk serology project.
    c. Steve Olsen, USDA, gave a report on a comparison of the serological response to administration of *Brucella abortus* vaccine RB51 using a needle-free injection system versus a standard needle-based injection.
    d. Keith Aune, Montana Department of Fish, Wildlife and Parks discussed the persistence of *Brucella* in the Northern Yellowstone environment and the disappearance of fetal carcasses in the same environment. The Subcommittee accepts the written report by Keith Aune and recommends its inclusion in the Proceedings of the 110th Annual Meeting.

3. Old Business
    a. Review the state of the science and determine the level of confidence of recently developed techniques for DNA finger-printing, genotyping *B. abortus*. This item will be removed from the agenda until further data is presented.
    b. Review the feasibility and capabilities for establishing a bulk milk brucellosis surveillance test for *B. melitensis* in goats. This item will be removed from the agenda until further data is presented.
    c. Review the feasibility and capability of matching DNA from sero-

positive blood to DNA from hair on corresponding back-tags of MCI reactors. This item will be removed from the agenda until further data is presented.

d. Update on outdoor research facilities checklist.

4. Other business

Review request, documentation and recommendations for the Committee.

a. Review of new instrumentation for use with FPA diagnostics (Instrument Equivalency Study).
b. Western blots and Yersinia was discussed.
c. Reevaluation of sensitivity and specificity of various tests was discussed.
d. Laramie Report was reviewed.

5. CLOSED SESSION:

a. Charge from Dr. Plumb regarding persistence of *Brucella* in the environment.
b. Decision on FPA technologies
c. USDA outdoor brucellosis research facility check list
d. Review of the USAHA Laramie Brucellosis Workshop Report.

The Subcommittee approves the Tecan Safire^2 and BMG PHERAstar instruments as equivalent to the Sentry 1000 FPA instrumentation.

The Technical Report and the Laramie Agenda from the USAHA Special Laramie Brucellosis Workshop were reviewed and accepted as written by the Committee.

The Subcommittee approved a recommendation that the outdoor animal brucellosis research facilities checklist be approved.

The Subcommittee report was unanimously accepted by the Committee.

The Committee approved the recommendation from the Scientific Advisory Subcommittee that USDA-APHIS-VS formally adopt the brucellosis outdoor research facility check list.

A Resolution was approved by the Committee and forwarded to the Committee on Nominations and Resolutions for consideration by the USAHA membership.

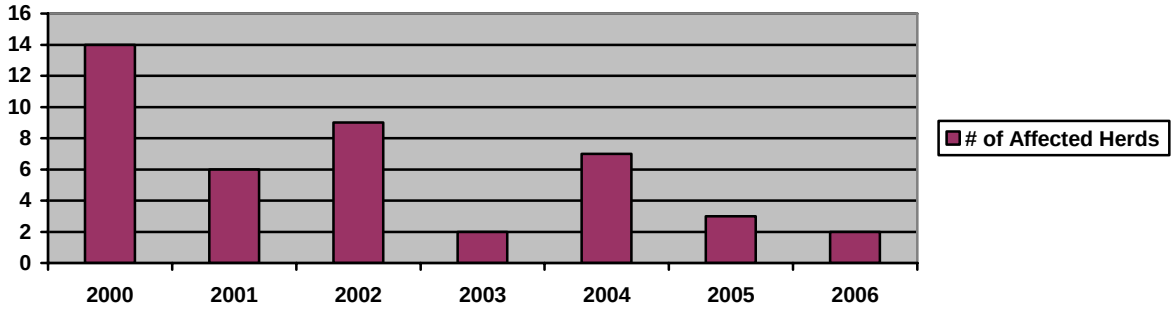
STATUS REPORT – FISCAL YEAR 2006 COOPERATIVE STATE-FEDERAL BRUCELLOSIS ERADICATION PROGRAM

Debbi A. Donch

Arnold A. Gertonson
Jack C. Rhyan
M. J. Gilsdorf
Veterinary Services

Fiscal Year (FY) 2006 was a year of aggressive efforts to attain final eradication of brucellosis while simultaneoulsy evaluating future brucellosis program needs once eradication of brucellosis from the nation's domestic cattle herds is achieved. Of the three states classifed as Class A for brucellosis, one state regained Class Free status and the other two states readied themselves to qualify for Class Free state status. Amending regulations, evaluating and formulating effective and efficient future surveillance plans, assessing ways to restructure the nation's brucellosis laboratory system for greater efficiency, and contemplating the future of the use of vaccination in the brucellosis program were all activities initiated in FY 2006. While additional brucellosis affected cattle herds were disclosed in FY 2006, significant strides toward final eradication were achieved.

A total of two new brucellosis affected cattle herds were disclosed in FY 2006. This compares to three new brucellosis affected cattle herds disclosed in FY 2005, seven new brucellosis affected cattle herds disclosed in FY 2004, two new affected cattle herds disclosed in FY 2003, nine new affected cattle herds in FY 2002, six in FY 2001, and fourteen in FY 2000. Both of the FY 2006 brucellosis affected cattle herds were disclosed in November 2005 in the state of Idaho, a state which had been classified as Brucellosis Class Free since February 1991. Both herds were depopulated with indemnity. The last reactor animal was removed the first week of December 2005. One FY 2005 brucellosis affected herd in Texas remained under hold order and test during FY 2006.



In mid-November 2005, Idaho identified the first of two brucellosis affected cattle herds. This herd was disclosed subsequent to a herd test as part of an MCI trace-back investigation. There were ten high-titered animals (eight reactors and two suspects) found on the herd test. Milk samples were obtained from the high-titered brood cows. *Brucella abortus* biovar 1 was confirmed by NVSL from cultures submitted from three reactor cattle. The affected herd was a beef herd located in Swan Valley (Bonneville County) in eastern Idaho. The herd was depopulated with indemnity. The most probable source of infection for this herd is brucellosis infected elk which migrate through this valley. The second brucellosis affected herd, a trace-out herd from the index herd, was identified in late November 2005. Classification as a brucellosis affected herd was based on reactor classified titers. Brucellosis program regulations define a brucellosis affected herd as "any herd in which any animal has been classified as a brucellosis

reactor and which has not been released from quarantine." With the disclosure of a second brucellosis affected herd, Idaho no longer met the requirements for Brucellosis Class Free state status. Of historical note - the last brucellosis affected cattle herd disclosed in Idaho was in May of 2002. This herd was located approximately sixty miles north of the current index herd. *Brucella abortus* biovar 1 was cultured from this herd which also had exposure to elk infected with the same biovar. Idaho originally attained Brucellosis Class Free state status in February 1991 and had maintained this status, having found only the single Brucellosis affected herd in 2002. Idaho is in the twelve-month qualifying period for Class Free state status. A pre-Class Free review was conducted in September 2006. Provided no additional brucellosis affected herds are found and all requirements are met, Idaho could qualify to regain Class Free state status in December 2006.

A brucellosis affected cattle herd disclosed in Hardin County, Texas in August 2005, remained under hold order and test throughout FY 2006. The herd plan stipulated herd depopulation if any additional reactor animals were found on subsequent whole herd serology testing. Brucellosis program standards (the UM&R) stipulate "three consecutive negative herd blood tests are required for release from quarantine (hold order), with the first negative herd blood test occurring 30-60 days after all reactors have been removed from the herd and slaughtered. The second of these tests must occur 180-210 days after all reactors have been removed and slaughtered. The third test (releasing test) must occur 365 days or more after all reactors have been removed and slaughtered." The final negative and releasing herd test was conducted in September 2006. Texas has completed a twelve-consecutive month period of finding no additional brucellosis affected herds and is currently conducting additional epidemiological evaluations in high-risk areas before applying for Class Free state status.

Wyoming officially regained Brucellosis Class Free state status on September 12, 2006. Throughout FY 2006, Wyoming worked to complete the required activities and implement the actions and recommendations made during the pre-Class Free review conducted in July 2005. Wyoming lost its Brucellosis Class Free state status in February 2004. Four brucellosis affected cattle herds were subsequently disclosed within a year's time; all were depopulated with indemnity. Wyoming initially obtained Brucellosis Class Free state status in October 1983. Brucellosis affected elk were identified as the most likely source of infection for the outbreak in 2004-2005.

Training continues to be a priority annual activity in the brucellosis program. Two brucellosis specific training courses were offered in FY 2006 – the Basic Brucellosis Epidemiology Course and the Designated Brucellosis Epidemiologist (DBE) Refresher Training Course. The Basic Brucellosis Epidemiology Course, attended by forty-three state and federal veterinary medical officers and animal health technicians, was taught in April 2006. The Basic Brucellosis Epidemiology Course is a three-day training event, with instructor-led lectures, facilitated discussions, practical exercises, and laboratory demonstrations. The purpose of the course is to provide training in the principles of the brucellosis eradication program, including the organism, the disease as it occurs in various species of animals, and detailed epidemiological considerations necessary to effect the efficient and rapid eradication of Brucellosis. The Designated Brucellosis Epidemiologist Refresher Training Course, conducted in August 2006 in conjunction with the Designated Tuberculosis Epidemiology Training Course, was attended by eighty-seven DBEs and DTEs. All DBEs are required to attend DBE refresher training once every two years to maintain their expertise in brucellosis epidemiology and be recertified for an additional two year period.

Brucellosis in the Greater Yellowstone Area:

A Greater Yellowstone Interagency Brucellosis Committee (GYIBC) Memorandum of Understanding (MOU) draft was updated (July 2006) to reflect Idaho's loss of brucellosis Class Free classification. It was signed by Secretary Johanns (2006) and is proceeding through the clearance process at the U.S. Department of Interior (DOI). After DOI concurs, the draft will be

resubmitted to the Governors of the Greater Yellowstone Area states (Idaho, Montana, and Wyoming) for their review and concurrence.

The Grand Teton National Park (GTNP)/National Elk Refuge (NER) Bison and Elk Management Plan and Environmental Impact Statement (EIS) final report is being drafted at this time. Publication of the final report is expected by early 2007. The cooperating agencies will have an opportunity to review the final report before it is published.

The Interagency Bison Management Plan (IBMP) cooperating agencies have been meeting to determine if changes in the IBMP are necessary to facilitate plan management operations. Adaptive management changes for operations can be made with the concurrence of all of the cooperating agencies. Montana initiated a bison hunt last year as part of the IBMP. The bison hunt was deemed a success (46 of 50 permits were filled) last year and the number of hunt permits has been increased to 140 permits for this year's hunt season.

APHIS VS personnel assisted IBMP bison management operations. Hazing operations (55) of 1317 bison were performed. All but 100 bison were successfully hazed back into Yellowstone National Park. Capture operations resulted in the capture of 995 bison. Nine bison tested brucellosis negative and were released. Eighty-six brucellosis sero-negative calves were placed in the brucellosis quarantine feasibility study facility. Fifty brucellosis sero-positive bison and 850 untested bison were shipped to slaughter. Under the IBMP protocol, bison may be shipped to slaughter if the Yellowstone National Park bison herd population is greater than 3000. Seven bison were lethally removed.

The GYA states (Idaho, Montana, and Wyoming) are proceeding, in consultation with APHIS VS, with development and implementation of individual livestock herd and individual elk herd unit plans to mitigate potential transmission of brucellosis from elk or bison to cattle. Idaho has completed its 2006 herd plans; implementation is planned for this year. Montana has completed its survey of livestock herds in the GYA and is performing a risk analysis of the individual livestock herds to determine management actions for inclusion in the individual livestock herd plans. Montana is reviewing its elk herd unit plans. Wyoming has a larger number of livestock herds and elk units affected by brucellosis. Wyoming is currently surveying livestock herd owners and development of individual livestock herd plans is ongoing. Wyoming has completed individual elk herd plans for four of the seven elk herd units of concern. Wyoming is also continuing statewide elk herd brucellosis surveillance using hunter collected blood samples. The northeast quadrant of Wyoming was surveyed in 2004. The southwest quadrant of Wyoming was surveyed in 2005 and the northwest quadrant is being surveyed in 2006.

Wyoming initiated a five year elk brucellosis test and removal of brucellosis sero-positive elk pilot project at its Muddy Creek feed-ground in 2006. Data gathered from this project will be evaluated to determine if test and removal will significantly reduce brucellosis sero-prevalence in those elk herds.

The study to determine the suitability of the Fluorescent Polarization Assay (FPA) and BAPA tests for brucellosis testing of elk sera is ongoing. Three state laboratories are working with NVSL to determine repeatability of test results. The study is expected to be completed in 2007.

APHIS VS personnel attended Wyoming Brucellosis Coordination Team, GYIBC, IBMP, and USAHA regional and national meetings, providing technical assistance and making presentations when requested.

Veterinary Services continued activities and involvement in several projects aimed at assessing potential effective *Brucella* control strategies for affected wildlife populations. These on-going developmental projects include the following studies:

- Engineered RB51 in elk: A study this year jointly conducted by Veterinary Services (VS) and Agricultural Research Services (ARS) examined the efficacy of engineered RB51 vaccine in elk. Although sample sizes were small, vaccinated elk had reduced incidence of abortion, uterine and mammary infection or maternal infection as compared to controls. Preliminary results of the study suggest the vaccine provides some level of protection against *Brucella abortus* abortion and infection in elk.

- Behavior studies in bison and cattle on exposure to bison and elk fetuses: These studies demonstrated mild to marked contact of bison and cattle with dead bison and elk fetuses placed in the environment. Analysis of the data is in progress.
- Test and treat strategy development:
  - Contraceptive studies: Two studies have shown at least three years infertility in bison following one injection of a GnRH immunocontraceptive vaccine.
  - Sustained release antibiotic treatment: In a limited pilot study, two weeks of therapeutic blood levels were obtained following a single injection.
- Serologic differentiation of *Brucella* and *Yersinia* infections: VS, with collaborators at LSU, ARS, and CFIA, is initiating a series of studies to examine serologic differentiation of *Brucella* and *Yersinia* infections in elk.

Brucellosis Program Surveillance Activities:

[The following surveillance statistics for the cattle brucellosis eradication program is based on data available as of September 15, 2006. Normal reporting time allowances for states to gather and submit monthly data preclude ascertainment of all data for FY 2006.]

Fiscal Year 2006 began and ended with 48 States and three Territories classified at Brucellosis Class Free state status, and two states classified at Brucellosis Class A state status. The two states classified as Class A at the beginning of FY 2006 were Texas and Wyoming. The two states classified as Class A at the end of FY 2006 were Texas and Idaho. From February 2006 to September 2006, three states, Idaho, Texas, and Wyoming, were classified as Class A. Idaho lost its brucellosis Class Free state status pursuant to the disclosure of two brucellosis affected herds in November 2005, and Wyoming met all requirements and officially regained Class Free state status in September 2006. Texas released its last known brucellosis affected herd from quarantine in September 2006, completing a consecutive twelve month period without disclosing any additional brucellosis affected herds. Thus at the end of FY 2006, 48 States and three Territories remain classified as Brucellosis Class Free state status.

Cattle inventories in the U.S. for FY 2006 were distributed as follows: 17.97% of all cattle and 16.82% of all cattle herds were located in the three Brucellosis Class A states; 53.34% of all cattle and 46.52% of all cattle herds were located in states that have held Brucellosis Class Free status for at least ten years (16.90% of all cattle and 17.17% of all cattle herds were located in states that have held Brucellosis Class Free status for five years or less; 36.44% of all cattle and 29.35% of all cattle herds were located in states that have held Brucellosis Class Free status for six to ten years). Approximately 28.69% of all cattle and 36.66% of all cattle herds were located in states that have held Brucellosis Class Free status for at least eleven years or more (with 15.25% of all cattle and 18.72% of all cattle herds residing in states that have held Brucellosis Class Free status for greater than 20 years).

Two brucellosis affected cattle herds, both in Idaho, were disclosed in FY 2006. The first affected herd was identified via Market Cattle Identification (MCI) surveillance testing, and the second affected herd was identified as an epidemiologic trace-out herd. The national herd prevalence rate for bovine brucellosis was 0.00021% in FY 2006. Per the Brucellosis Emergency Action Plan (BEAP) recommendation, both brucellosis affected herds were depopulated with indemnity and thorough epidemiologic investigations were completed disclosing no additional brucellosis affected herds. In addition, trace exposed test negative cattle were depopulated and indemnified as well.

Maintaining brucellosis state status focuses on continual surveillance activities. Two primary surveillance activities are conducted for bovine brucellosis, Market Cattle Identification (MCI) testing and Brucellosis Milk Surveillance Testing (BMST). During FY 2006, APHIS tested approximately 7.921 million head of cattle under the MCI surveillance program. Brucellosis program standards require testing of a minimum of 95% of all test-eligible slaughter cattle. In FY 2006, approximately 96.04% of all test-eligible slaughter cattle were tested. First-point testing at livestock markets is required in Brucellosis Class A states. Twelve Brucellosis Class Free states continue to conduct first-point testing at markets to enhance their surveillance activities.

Brucellosis program standards require a minimum of 90% successful traceback of all MCI reactor cattle and a minimum of 95% successful case closure. In FY 2006, approximately 97.2% of all MCI reactors were successfully traced and investigated resulting in successful case closures. Approximately 868,500 additional head of cattle were tested on farms or ranches during FY 2006, bringing the total cattle tested for brucellosis in FY 2006 to approximately 8.790 million head. BMST surveillance is conducted in all commercial dairies – a minimum of two times per year in Class Free states and a minimum of four times per year in Class A States. Suspicious BMSTs are followed up with an epidemiologic investigation. 2005 National Agricultural Statistics indicate there were 78,295 dairy operations in the U.S. There were approximately 164,000 BMSTs conducted in FY 2006; approximately 186 of those BMSTs yielded suspicious results after repeated screening (repetitive BRT and/or HIRT). All suspicious BMSTs in FY 2006 were confirmed negative by subsequent epidemiologic investigations and additional herd testing.

There were approximately 4.423 million calves vaccinated for brucellosis in FY 2006. The national calfhood vaccination policy recommends proper calfhood vaccination in high risk herds and areas and whole herd adult vaccination when appropriate in high risk herds and areas. Elimination of mandatory vaccination in all states is also recommended.

Brucellosis program activities throughout FY 2006 clearly demonstrate the continued commitment of the state-federal cooperative brucellosis eradication program to achieve final eradication of brucellosis from the United States domestic cattle, bison, and swine herds. Aggressive actions and the resolve to address difficult issues have set the stage for all states to be classifed at Brucellosis Class Free state status by the end of FY 2007. As eradication nears, focused, efficient, and effective surveillance becomes paramount to the integrity of a national brucellosis-free classification for the United States.

PLANNING CATTLE BRUCELLOSIS SURVEILLANCE

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Although the objective of the bovine brucellosis eradication program is clearly to eliminate all cattle brucellosis from the United States, that doesn't clarify what the objective of surveillance should be; unless we agree to test every animal every minute each day with the intent to slaughter any that react. Surveillance objectives state how confident we need to be in detecting infection at a defined (non-zero) prevalence level. I think you will appreciate that the prevalence level we can detect is exceptionally low, but we cannot rely on statistics per se to finally declare that we have completely eliminated brucellosis. Ultimately, that declaration will be based on our decision that the estimated prevalence is low enough for long enough that we don't think there is brucellosis here.

As of this year, 48 States are listed as Class Free based on the brucellosis regulations. 34 of these States have been Class Free for 10 or more years while 22 of those States have been Class Free for 20-plus years. Yet, the intensity of surveillance in Class Free States has remained at the same level for the past few decades. One has to wonder whether surveillance should continue at the same intensity forever or if we are at a point where opportunities may exist to modify surveillance in Class Free States.

There are two general categories of findings we present: findings related to sampling activities and findings related to laboratory testing and information management I want to emphasize that sampling findings only apply to Class Free States that do not border the Greater Yellowstone Area. Our team has not been asked, nor did we seek, to be involved in evaluating surveillance activities in the GYA. Instead, we had our hands full in the past months analyzing surveillance for the great majority of the U.S. that is considered Class Free and at low risk of acquiring bovine brucellosis from wildlife.

Sampling findings point to redundant surveillance activities and apparent surveillance imbalances. First, surveillance evidence accumulated in Class Free States provides adequate statistical confidence in their freedom from brucellosis. Statistical analysis cannot prove zero, but the sampling evidence suggests a low probability that brucellosis exists among Class Free

States. Next, we find little benefit from conducting both slaughter surveillance and BRT surveillance among dairies. BRT is a good herd-level assay and provides a high degree of confidence about the status of dairy herds in such States. Along this same line, surveillance is currently biased toward finding affected dairy herds, despite evidence suggesting dairies face a lower risk of brucellosis infection compared to beef herds. Finally, a lot of first point testing is still going on in Class-free States, but much of this sampling is redundant with slaughter sampling.

Multiple strategies for detecting infection are not a bad thing for surveillance. But, to be useful, different strategies have to target different subpopulations. For beef herds, surveillance includes slaughter testing of all cull cows and bulls as well as 1st point testing of cattle sold through livestock markets. But, many of the cattle that enter livestock markets are cull cows sold for slaughter. Testing cows at market and again at slaughter buys very little additional information, especially because test results correlated.

For dairy herds, the BRT is highly effective for evaluating whole herds of cows. Some cows included in BRT, however, are sampled at slaughter.

For quite a while, the number of brucellosis-affected dairies has been substantially less than the number of affected beef herds. Risk of importing brucellosis into a herd is directly related to the number of affected herds that might sell (undetected) animals to the herd. After at least five years of surveillance within a Class Free State, the risk of importing brucellosis becomes the most important consideration for surveillance. There have been no affected dairies detected since 1999, but brucellosis continues to be detected among beef herds, albeit at a low rate.

Crude estimates of how approximately $30 million annually appropriated for brucellosis surveillance is allocated across the three main surveillance sampling activities suggest, in the end, that we spend a lot on slaughter and 1st point testing. Estimates include collection, transportation, testing and investigation costs estimated on a per sample basis. Estimates also include personnel, equipment, materials and overhead costs. These funds only represent annual Federal expenditures. Overall, States provide as much or more funding to support these activities.

There are a couple of reasons why slaughter surveillance of beef herds is problematic. First, most beef herds in the U.S. are small; on average 25 cows or less. In fact, according to NASS, there are fewer than 5000 herds with more than 500 cows per herd among Class Free States. With respect to culling management, a smaller proportion of cows are culled from smaller herds than from larger herds. In any given year, herd-level sensitivity measures the probability of detecting a herd given that it contains one or more infected cattle. Because only examine a small number of cattle at slaughter per herd, herd-level sensitivity is low in small herds. Furthermore, because the great majority of beef herds are small, the overall herd-level sensitivity is low.

Number one hazard for Class Free States is importation of new infection into those States. Estimates imply that a typical Class Free State might import one or more infected cows once every 12 to 100 years.

We developed several statistical models to estimate herd prevalence of brucellosis in Class-free States based on slaughter surveillance results. One challenge was making inferences about herd prevalence from the individual animal samples collected at slaughter. One model's predictions – based on negative surveillance evidence from beef herds in the Class Free States – suggest that, by five years, we are 95% confident there are fewer than 3 affected herds among all more than 600,000 herds in Class Free States. But, amount of surveillance conducted at slaughter, combined with the risk of introducing infection into one or more of these States, results in a leveling-out in estimates beyond 5 years.

Within two years of achieving Class Free status, our model suggests we are 95% confident that fewer than 2 affected herds exist among 75,000 dairies. Why would slaughter surveillance provide such confidence for dairies while taking much longer for beef herds? Mostly, because dairy herds, on average, are larger – and cull a larger fraction of their herds each year – than beef herds. So, slaughter surveillance more statistically efficient in dairy herds than beef herds.

BRT is probably the best test ever developed for a national eradication program. Brucellosis ring testing dairies once per year for 5 years provides nearly 98% confidence that one or fewer affected herds exist in Class Free States. If two rounds of BRT conducted each year for 5 years, this confidence increases to over 99%. But, improvements in confidence come at a cost.

For example, we gain almost 98% confidence across five years using one round of BRT and the Federal cost is approximately $1.6 million per year. But, two rounds per year (current requirement for Class Free States) results in just over a 1% gain in confidence while costing twice as much as one round. Reduced cost efficiency is most evident when consider that 3, 4 or 5 rounds per year hardly improve our confidence but cost another $1.6 million each round.

Redundancy and imbalanced intensity of surveillance apply to dairies. Two rounds of BRT per year in Class Free States provide 99% confidence of detecting one or more affected dairies (among all Class Free States) after 5 years of surveillance. Yet, combining BRT and slaughter surveillance improves ability to detect a single affected herd by less than 1%. Sampling dairy cows at slaughter that probably sampled in the BRT and double sampling amounts to redundancy – at least after five years of negative results.

Also, recall slaughter surveillance of beef herds in Class Free States provides 95% confidence in detecting 3 or more herds. Slaughter surveillance of dairy herds in these States provides 95% confidence in detecting 2 or more herds while BRT alone provides 99% confidence in detecting 1 or more affected dairy herds. So, scrutiny of dairy industry with either slaughter or BRT surveillance is greater than scrutiny of beef industry. This imbalanced intensity of surveillance seems less appropriate when we factor in the much lower likelihood that dairy herds will become affected.

Arguably, 1st point testing is responsible for most of the progress we've made in eradicating brucellosis. But, we currently spend a lot on 1st point testing. 13 States (including the two Class A States) reportedly still do some 1st point testing. This testing is not required in Class Free States, although it is recommended for a few years after achieving this status. Returning to our theme of redundancy, however, there are a percentage of 1st point samples in Class Free States come from cows or bulls likely slaughtered soon after being offered for sale at livestock markets.

Our evaluation also examined the crucial role laboratory testing plays. The history of diagnostic serology for brucellosis is somewhat responsible for the situation today. As new diagnostic tests have been approved, they have been added to testing protocols, but older (more trusted) tests have also been retained. It is difficult to compare test results across laboratories because many different serologic protocols are used. Furthermore, the use of multiple confirmatory tests is difficult to explain theoretically. Analyzing surveillance data at a national level is always daunting. But, brucellosis information systems make task nearly impossible. Differences between States make aggregating information at regional or national level extremely difficult. Complaints about our brucellosis surveillance system have circulated for years, so this finding not a surprise.

About one year ago we surveyed designated brucellosis epidemiologists about the laboratory protocols used for brucellosis surveillance in their States. Typically, brucellosis testing involves an initial screening test on all blood samples collected. Any positive samples on the screening test are subsequently re-tested using confirmatory tests. There are a number of tests that may be used for screening blood samples based on our survey. There are also a number of confirmatory tests conducted on brucellosis blood samples based on our survey.

So, what are we trying to do with our brucellosis serology protocols? It is sensible to conduct testing in series. Screening all samples and retesting positives reduces chance of classifying uninfected cattle as false positive. Improved specificity is appropriate because of the generally low prevalence of brucellosis. Nevertheless, running multiple confirmatory tests actually confuses the objective of diagnostic protocol. If any confirmatory test is suspect or reactor range, the animal is considered positive. Such an approach, called parallel testing, increases the chances of a false positive result. Although this improves sensitivity of confirmatory test, it never improves sensitivity beyond the performance of the screening test! If miss infected cattle at the screening stage, can't make it up with a really sensitive confirmatory stage. Overall, running multiple confirmatory tests could result in a protocol that theoretically has lower sensitivity AND specificity than just running a single test! Furthermore, unpredictable performance of variable protocols used in the 80+ labs currently approved to conduct brucellosis testing makes it difficult to interpret and monitor performance.

If want to monitor performance of labs, then examine the proportion of positive samples each lab reports across time. In diagnostic terminology, this is a form of repeatability.

Generally, animal population tested in a particular lab across time should be somewhat stable and, correspondingly, so should the proportion of positive samples. We were able to examine repeatability for one State's cattle population because that State captured all individual animal ID information in their testing database. That analysis illustrates that the number of cattle tested from this State has declined across time and the proportion of cattle found BAPA and card-positive each year is variable. If all cattle tested from this State are uninfected (and we strongly believe they are) then these are false-positives.

What we need to ask is: does this variability in test-positive frequency make sense? The answer depends on what we think the specificities of the BAPA and Card tests are. Research on this subject is confusing: suffice it to say we see a need to establish expectations about the minimum proportion of samples that should be found positive. Expectations should be quantitatively monitored across time to determine if they are being met. Failure to meet the expectations could generate further investigation as to cause and improve our understanding of diagnostic test performance in the long run.

Another measure of lab performance is how results compare between different labs. This is a measurement of reproducibility. Results show testing conducted at livestock markets across four different States. There is variability in the proportion of samples found to be suspects or reactors. The point of this finding, as well as the previous finding, is not to explain why results may vary across time or labs, but to point out that this variability should be monitored and, ultimately, controlled within specific limits so that testing conducted by labs can be appropriately compared and contrasted. Only by understanding the reasons for different results can we hope to gain a better appreciation of our surveillance systems. But, the current status of lab protocols in our brucellosis program makes it nearly impossible to gain this understanding.

BRT results since 1950 demonstrate the stability of reported BRT suspicious herds per year, between roughly 1970 and 1990. Also, there is a sharp decrease in reported positive BRT samples around the mid-1990's corresponding to the introduction of RB51 vaccine. A drop in BRT-positive results was expected as we removed Strain 19 from the picture. But, there were fewer than 50 BRT positive results reported into the national database by 2003. If assume there are no affected dairy herds in the U.S., then it is estimated that 50 out of a minimum of 160,000 BRT's are false-positives, or 1 per every 3200 tests run. A specificity of 99.97% is totally out of line with reported specificities for this test. For example, published research suggests the specificity of the BRT may be less than 90%. Are BRT positive samples not being reported and/or investigated simply based on the assumed absence of infection among dairies? Are epidemiologists inserting too much judgment into their interpretations? We need more investigation, but we think performance standards could be useful to ensure consistent interpretation of these tests.

There needs to be a mechanism for summarizing surveillance activities at national level, but current information systems do not support the level of detail needed. Databases are not consistently used; we have nearly 50 distinct entities completing data entry using approaches that work for them, but don't support accumulation of the data or comparison of the results across States, regions or the nation. In our review of national database information, we find substantial variability in the data fields entered, as well as the choice of entries. From a surveillance perspective, the absence of complete information on animals with test-negative results is a real problem. At a practical level, expend lots of effort ensuring collection of animal ID only to throw that information out if the test comes back negative.

Current brucellosis information system is highly variable in quality and usefulness; result is a system that is inconsistent and cannot be monitored to ensure that our program is accountable. We can do better. There are 13 labs that account for about 80% of all blood samples processed each year in the U.S. Currently, there are more than 80 labs approved to conduct official brucellosis serology. There is a State-Federal working group looking into consolidation of approved brucellosis laboratories. It seems that reducing the number of labs – while increasing support for data entry – could facilitate better standardization and monitoring.

There are only 40 or so slaughter plants (as represented by the larger circles in this diagram) that are responsible for processing more than 95% of the cull cows and bulls in the U.S. per year. Nevertheless, there are approximately 500 slaughter plants shown here that process some number of cows and bulls each year. Economies of size suggest we could concentrate on

collecting quality samples and information from the larger slaughter facilities. If those samples were processed in a limited number of laboratories using the same testing protocol and all available data were consistently entered into a database, then the efficiency of surveillance would likely improve.

In summary, our evaluation suggests that eliminating redundancies in surveillance among Class Free States might improve efficiency by providing sufficient statistical confidence for less than the current investment. It also suggests that we need to balance surveillance better between the beef and dairy sectors.

Surveillance also depends on laboratory protocols and data entry. We can improve these by developing quantitative performance standards and consistently applying these standards in our work. Finally, I want to emphasize that our findings, while focused on efficiency, are not independent of effectiveness. It does us no good to become more efficient if the quality of surveillance evidence collected is reduced. Nevertheless, we think both efficiency and effectiveness of surveillance can be improved.

BRUCELLOSIS IN YELLOWSTONE BISON: AN INDIVIDUAL-BASED SIMULATION MODEL OF VACCINATION STRATEGIES

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Yellowstone National Park (YNP) is a partner to the Interagency Bison Management Plan (IBMP) to "maintain a wild, free-ranging population of bison and to address the risk of brucellosis transmission to protect the economic interests and viability of the livestock industry in the state of Montana" (NPS 2000). The IBMP addressed vaccination as a potential action and the National Park Service (NPS) is now proposing to conduct remote vaccinations of wild, free-ranging bison within the boundaries of YNP. The complexity of implementing a brucellosis vaccination program requires the assessment of a variety of alternatives. Understanding brucellosis epidemiology and evaluating potential vaccine control strategies are necessary components of the development of a bison vaccination program. To evaluate management alternatives aimed at reducing brucellosis infection in Yellowstone bison, we developed an individual-based model (IBM) to predict how brucellosis in YNP bison might respond under each alternative strategy.

The objective of the vaccination program is to reduce the risk of brucellosis transmission to livestock outside YNP by decreasing brucellosis infection in the Yellowstone bison herd. Brucellosis seroprevalence has been estimated to fluctuate between 40-60% in YNP bison during the past 20 years. This range of infection was simulated prior to the analysis of each vaccination scenario. Model scenarios included vaccination of female calves and yearlings captured during boundary management operations, combining remote vaccination using biobullet delivery (Olsen et al. 2006) with boundary vaccination of female calves and yearlings, and vaccinating all female bison during boundary operations and as targets for remote delivery. Under each alternative, bison captured at the boundary would be tested and seropositive bison would then be removed.

We chose an individual-based modeling approach that captures the variability between individuals and measures their response to both the disease and vaccination. The IBM tracked information on each female bison born into the population. The model used both yearly and daily times steps. The yearly time step components involved mating, natural mortality, exposure to *B. abortus* via elk, and effects of NPS management operations (testing and then removing seropositive bison at boundaries). The daily time step detailed the processes leading to shedding and transmission of *B. abortus* among Yellowstone bison. Male bison were included in yearly

outputs, but were not a focal component of the model. Demographic, life history, and management related information (age, sex, disease status, reproductive status, vaccination status, and management removal) were recorded for each female bison modeled.

Modeled bison were initially assigned a disease status (Susceptible, Infected, or Latent) based on estimates derived from Yellowstone bison seroprevalence data. Susceptible bison were those that had never been exposed to *B. abortus*. Infected bison shed *B. abortus* at a high probability (.96 derived from experimental studies, Olsen et al. 2003) during their next pregnancy. These infected bison then enter a latent class with a low probability of shedding *B. abortus* during future parturition events. Temporal changes in the disease classes of individuals were used to predict the disease status for the overall population. Individuals changed their disease class based on events (i.e., exposure, vaccination) and rules associated with their current state (i.e., disease class, pregnancy status, vaccination status).

The model included two types of infectious events for horizontal transmission; *Brucella* induced abortions, and infectious live births. We assumed that both events had equal transmission potential. We also assumed that infected bison did not fully recover from the disease. These animals had a low probability of shedding the bacteria in future pregnancies. In situations where latent cows recrudesced and shed *B. abortus* during an infectious live birth, their calves became infected through vertical transmission at a specified probability (.66, from Gross et al. 1998).

In the model, vaccinated susceptible bison were classified as vaccine-protected and were prevented from shedding *B. abortus* based on the assigned efficacy of the vaccine. These bison remained vaccine-protected until they were exposed to the field strain. Vaccine-protected bison subsequently exposed to field strain *B. abortus* remained protected subject to the duration assigned to the vaccine. Vaccine efficacy was modeled as the proportion of susceptible bison receiving the vaccine that become vaccine-protected (do not shed *B. abortus*). Likewise, the duration of vaccine protection was modeled as the probability of remaining vaccine-protected if exposed to field strain *B. abortus*. This strategy allowed for altering the level of protection in future exposures following vaccination. When field exposure overwhelmed the protection of the vaccine, the bison became infectious. A vaccine delivery parameter was used for alternatives involving remote vaccination. This was the proportion of targeted bison in the population that were likely to receive the vaccine. These bison entered the vaccine-protected class based on vaccine efficacy.

Preliminary data were compared from outputs simulating each of the three alternatives at intermediate levels (.5) of the following vaccination parameters: vaccine delivery, vaccine efficacy, and duration of vaccine protection. Combining boundary and remote vaccination of all female bison resulted in the greatest seroprevalence decline over the 30 year simulation period (Table 1). This alternative also resulted in a larger proportion of vaccine-protected bison (Table 2) compared to the other two alternatives.

This model was developed to better evaluate vaccination alternatives for reducing brucellosis infection in the Yellowstone bison population. The most effective strategy was to focus vaccination efforts on all female bison during boundary management and remote vaccination. Expanding to all female bison will include every bison believed to be important in the maintenance of the disease. In addition to providing the greatest seroprevalence decline for vaccination investment, this strategy maximizes the potential for bison to receive multiple vaccinations throughout their life.

There is a need for multiple indicators to measure the effectiveness of this (or any) vaccination strategy. Seroprevalence is an attractive indicator because it is obtainable without great difficulty and does not require killing the animal. Nonetheless, it should be monitored in combination with other indicators. Seroprevalence indicates a history of exposure and does not provide a complete picture of how bison may be responding to vaccination. Vaccine-protected bison that are subsequently exposed to field strain *B. abortus* may be protected from infection but will react positively on serologic tests. As a result, there will be a delay in seroprevalence decline resulting from the removal of vaccinated bison that have been exposed. Serology tests should be combined with culture work to estimate the proportion of infectious animals that react positively on serologic tests. Linking culture tests conducted on bison removed during

management operations with their serology will provide a more accurate understanding of how bison are responding to the vaccination program.

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Table 1. Seroprevalence declines for each vaccination alternative modeled for 30 years

Management Alternatives	**10 Yrs**	**20 Yrs**	**30 Yrs**
Boundary vaccination of female calves and yearlings	44%	37%	30%
Boundary and remote vaccination of female calves and yearlings	39%	32%	26%
Boundary and remote vaccination of all female bison	30%	17%	13%

Table 2. Percent bison vaccine protected after 30 years

Management Alternatives	**Percent Vaccine Protected**
Boundary vaccination of female calves and yearlings	44%
Boundary and remote vaccination of female calves and yearlings	39%
Boundary and remote vaccination of all female bison	30%

APPLICATION OF A REAL-TIME PCR ASSAY FOR *BRUCELLA ABORTUS* IN WILDLIFE AND CATTLE

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In 2003 we reported on the development of a hybridization probe-based real-time polymerase chain reaction (PCR) assay for *Brucella abortus*, and the comparison of that assay to SYBR Green and hydrolysis probe assays (Newby, et al., 2003. *Appl. Environ. Microbiol*. **69**, 4753-4759). We found the hybridization probe assay to be of superior sensitivity and selectivity, and have been using the assay for the past 3 years to evaluate its utility in efforts to understand and eliminate brucellosis in large ungulates in the greater Yellowstone area of the United States. While all real-time PCR assays can provide some quantitative estimate of the amount of starting template present in the reaction (which can be used to estimate pathogen numbers in the original sample), SYBR Green and hybridization probe-based assays also permit post-amplification analysis of product specificity by melt curve analysis.

Our initial efforts were focused on establishing a method for field testing of animal blood samples using the real-time assay. The hybridization probe assay is ideally suited to the Idaho Technology, Inc. Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.; Figure 1).

Figure 1. Field portable real-time PCR instrument

This instrument uses glass capillaries, rather than plastic tubes to contain and monitor the PCR reaction, and is based on the same technology licensed by Idaho Technology to Roche (sold as the LightCycler® in various configurations). We envisioned blood as the ideal sample for such testing, in line with current serological tests performed to screen animals in test and slaughter programs. In the laboratory, our assay could detect Brucella DNA in as little as 15 minutes (20 cycles; more time was required to achieve detection of the limit of detection, 7.5 fg – equivalent to 2 genomic copies), but the overall time for analysis must also factor in time necessary for DNA extraction from blood or other samples. In practice, we have been able to analyze a full instrument load of 32 samples and controls after DNA extraction from blood within 2 hours. We performed a field test of our assay and extraction procedures on elk at the Idaho Fish and Game Department's Wildlife Health Lab in Caldwell, Idaho, in November, 2003. Environmental conditions, particularly cold temperatures, were found to challenge the instrument's ability to function properly (extended cycle times due to increased heat demand, and loss of computer control; these effects were particularly noticeable at temperatures below 0°C). We also found that it was difficult to avoid sample cross-contamination when performing DNA extractions in the field with wind and animal movement contributing to airborne particulates. Operation in a trailer or other enclosure during the winter would therefore be essential, and recommended during warmer seasons if chute-side testing is desired.

We have subsequently tested a wide range of samples, including animal blood, reproductive tract tissues, milk, vaginal swabs, mammary secretions, amniotic fluid, soil, and bacterial cultures. Laboratory testing of blood samples from cattle, bison, and elk suggest that application of the assay for blood testing might be best suited to cattle, as results in bison have largely been negative (no positive results compared to culture results from the same animals), and the sampling for elk has been too limited to draw definitive conclusions. Testing in cattle applied to samples from a vaccine challenge study indicated the assay could detect *B. abortus*, but that blood samples gave best results when fresh. Extended storage of frozen samples led to degradation and loss of signal using the assay. Blood collection into anticoagulants such as citrate or EDTA gave better results, as any coagulation led to unacceptable carry-over of heme (a well known PCR inhibitor) into the DNA extract.

Table 1 summarizes the results from these tests. All blood samples required use of some method of DNA extraction. We have experimented with a number of commercial kits for blood extraction, and found that products developed for forensic DNA analysis were suitable under some circumstances. In general, blood was a difficult test material. Tissue samples also required extraction, but we obtained positive results from samples that were also known to be culture positive. Amniotic fluid and milk appear to be samples that can be easily tested with this assay, as no DNA extraction was required. Even whole milk samples could be used directly, although it is likely that some inhibition occurred due to the presence of fats in the sample. Soil

samples from areas frequented by bison in Yellowstone National Park were also tested, and gave variable results. Samples which tested positive were not consistently positive upon subsequent retesting. Since none of these samples were culture positive, it may be that these were false positives, or that the amount of Brucella present in the original samples was exceedingly low (below the level necessary to produce a colony on an enrichment plate).

We have also used the assay extensively to confirm the identity of reference strains in our culture collection, and to test presumptive isolates. In several cases, identification of presumptive *B. abortus* isolates using the assay was later confirmed by testing at the National Veterinary Services Laboratory.

In summary, we have found this assay to be useful in detecting *B. abortus* in a wide range of animal samples. While blood samples have proved to be difficult to test, tissues, and particularly amniotic fluid and milk are easily analyzed for the presence of the pathogen (the latter without need for a DNA extraction step). The assay is also useful for rapidly screening presumptive isolates of *B. abortus*, with the caveat that it cannot discriminate between virulent strains and the current vaccine strains S19 and RB51, although we have not examined in detail whether post-amplification melt curve analysis might provide such discrimination.

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Table 1. Sample types tested using the *B. abortus* specific real-time PCR assay.

Sample Description	*B. abortus* Detected	Culture Status	No. of Samples	Comments
Brucella Culture collection	+	+	97	Questionable cultures flagged
Blood (cattle)	+ (2)	+	7	RB51 challenge study
Blood (bison)	-	-	89	seropositive; 3 culture positive
Blood (elk)	-	-	18	seropositive
Mammary gland (bison)	+	+	1	
Mammary gland (elk)	+	+/-	1	seropositive
Mammary secretion (elk)	-	-	2	seropositive
Milk (cattle	+	+	3	presumptive positives confirmed; no DNA extraction necessary
Secondary sex organ	+	+	1	
Amniotic fluid	+	+	2	No DNA extraction necessary
Soil	+	-	13	Negative on re-test

THE EPIDEMIOLOGICAL CHALLENGE OF EVALUATING BRUCELLOSIS SEROPREVALENCE IN FREE-RANGING ROCKY MOUNTAIN ELK IN MONTANA

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Animal and Plant Health Inspection Services

Serologic surveillance for bovine brucellosis in Montana's elk populations occupying the area surrounding Yellowstone National Park was first performed in 1981. Additional surveys were conducted periodically thereafter and in the early 1990's Montana Fish, Wildlife and Parks (FWP) began regular collection and testing of serum collected from hunter-harvested elk in this region. Results from these surveillance efforts have indicated that brucellosis seroprevalence is low (< 2%), however, culture results from five research animals in 1988, one hunter-killed animal in the early 1990's and an aborted fetus in 2005, confirm the presence of *Brucella abortus* biovar 1, and the mechanism for it's transmission, in the elk populations of the Greater Yellowstone Area (GYA).

In 2002 the Montana Elk-Brucellosis Management Plan (MEBMP) was drafted, aiming to standardize brucellosis surveillance efforts, integrate surveillance with the state Elk Management Plan, and provide guidance for enhanced surveillance and management of elk. Under the MEBMP, three elk management units (EMU) were established within the GYA: Emigrant, Gallatin and Madison. The plan recommended comprehensive sampling be performed once every three years from each unit with the establishment of an Epidemiological Review Team (ERT) in response to increasing trends in seroprevalence over three consecutive sampling periods or a seroprevalence >5% in any single sampling period.

In 2004-05, seroprevalence was determined to be 6.9% in the Madison EMU. As the 5% seroprevalence limit was exceeded in this unit an ERT was established to investigate these findings and new annual surveys were initiated to evaluate seroprevalence in all three EMU. Blood collection kits were mailed to permit holders, delivered to cooperating landowners, and placed in kiosks located at major hunter access points. Educational information was distributed to the media, local sporting goods stores, and area sportsmen's groups in efforts to inform hunters of the process for collecting and submitting samples. Due to a clerical error additional blood collection kits were mailed to permit holders in the Pioneer Mountains, an area outside the GYA. Since brucellosis surveillance of elk from this area had not occurred previously, samples received were tested in an effort to further increase knowledge of the distribution of *B. abortus* in Montana.

Samples collected in 2005-06 were submitted to the Montana Veterinary Diagnostic Laboratory and serological screening was conducted using Card, Rivanol, Complement Fixation (CF) and Fluorescence Polarization Assay (FPA) tests. Buffered Acidified Plate Assay (BAPA), Standard Plate (SPT) and Standard Tube (STT) Tests were performed on all positive or suspect cases identified by the screening tests. Unexpected seropositive samples from the Pioneer Mountains (4/35 or 11.4%) and elevated seroprevalence in the Madison EMU (24/137 or 17.5%) prompted a review of test data and a more detailed investigation of brucellosis in elk in the GYA.

Certain strains of bacteria including *E. coli*, *Salmonella,* and *Yersinia* are known to cross-react in serologic tests designed for *B. abortus*, leading to false positive results. To investigate potential cross-reactivity and further evaluate the reliability of the serologic findings, all available serum from positive and suspect samples originating from the Pioneer Mountains and the Madison EMU collected between 2004 and 2006 was submitted for the Western Immunoblot (WB) test. While WB is not an approved regulatory brucellosis test, is time-consuming, requires subjective interpretation and is currently only performed by a single laboratory in the US, it is considered to be highly reliable as a means of differentiation and is commonly used as a research tool. Results from the WB indicated widespread cross-reaction with the bacterial strain *Yersinia enterocolitica O:9*. Test results from the Pioneer Mountains and Madison EMU surveys were re-evaluated and

upon consideration on the WB results several samples formerly considered positive for brucellosis were reclassified as negative by the state Brucellosis epidemiologist. Seroprevalence was recalculated for the Pioneer Mountains and the Madison EMU and found to be 0 % and 1.93 % respectively. Results for Madison EMU are shown in Table 1.

Cross-reactivity in serologic tests for brucellosis, until recently, has not been considered a significant issue in elk from the GYA. Seroprevalence for brucellosis was considered to be low, < 2% in most years, and brucellosis had only been detected within the GYA. The significant increase in seroprevalence observed over the last two years as well as detection of seropositive animals a considerable distance from the GYA raised questions about the interpretation of serologic results. In Montana, WB was used on seropositive and sero-suspect samples to enhance our ability to interpret serologic findings and determine if cross-reactivity was indeed an issue. The detection of *Yersinia* antibodies offers an explanation for the sudden increase in apparent seroprevalence observed in recent surveys.

Based on these findings, the Montana laboratory testing protocol for free-ranging elk for the 2006-07 sampling period has been modified. Screening of all serologic samples will be performed using SPT, Rivanol, and FPA tests. CF, WB and cELISA will be used as supplemental tests for all reactors or suspect samples.

Exposure of elk to *Y. enterocolitica O:9* in the GYA and its potential impact on elk herds remain unknown and warrants further investigation. False positive results due to cross-reactivity demonstrate the challenges of evaluating serologic results. Although WB test results indicate that seroprevalence of brucellosis in the GYA has not changed significantly since the early 1980's, the collection of a culture positive aborted elk fetus in the Madison Valley in 2005 confirms brucellosis is present, the potential of exposure to cattle herds still exists and supports continued efforts to understand and manage for brucellosis in elk populations of the GYA.

Table 1. 2004-2006 Madison Elk Management Unit Brucellosis Serology Results and reclassified results based on WB findings indicating cross-reactivity with *Yersinia*.

Total number of samples	311
Number of positive samples based on serology only	36
Percentage of sample seropositive prior to Western Blot	**11.57**
Number of samples submitted for Western Blot	34*
Number of samples positive for *Yersinia* only (Western Blot)	30
Number of sample positive for *Yersinia* and *Brucella* (Western Blot)	4
Percent positive after reclassification	**1.93**

* Extra serum was not available for retesting two seropositive samples; Western Blot was therefore not performed.

AN UPDATE ON THE INTERAGENCY BISON MANAGEMENT PLAN FOR YELLOWSTONE NATIONAL PARK AND MONTANA.

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Agencies participating in the Interagency Bison Management Plan include the National Park Service, USDA Forest Service, USDA Animal and Plan Health Inspection Service, Montana Department of Livestock, and Montana Department of Fish, Wildlife and Parks. The goals of the IBMP are to reduce the risk of transmission of brucellosis from bison to cattle; to reserve a viable, wild population of Yellowstone bison; to maintain Montana's brucellosis Class Free status; and to protect private property. The IBMP employs several bison management tools including hazing, captures, testing, shipment to slaughter and lethal removal in order to manage risk. The bison population entered winter 2005-2006 at approximately 4,900 animals, during the winter and spring seasons, brucellosis risk management practices removed 915 animals. The population also declined an additional 587 bison by other causes (Table 1). Results from serological tests

conducted on samples taken at slaughter facilities disclosed that 43% of bison tested sero-positive for brucellosis.

Table 1. Summary of Interagency Bison Management Plan activities, 2005-2006.

INTERAGENCY BISON MANAGEMENT PLAN - FEDERAL FY 2006 (October 1, 2005 thru July 26, 2006)							
MANAGEMENT ACTIVITY	LOCATION			TOTALS			
	West Boundary --outside park--	North Boundary --inside park--	North Boundary --outside park--		% of total removals and mortalities		
Brucellosis Risk Management							
Hazing							
Number of hazing operations	24	87	31	142			
Mortality during hazing activity	2	0	0	2			
Capture							
Number of capture operations	4	8	0	12			
Total Bison Captured	59	1249	0	1308			
Released (Not tested)	9	305	0	314			
Transported to Slaughter (Not tested)	50	838	0	888			
Transported to Slaughter (Tested)	0	11	0	11			
Capture Pen Mortality	0	8	0	8			
Lethal Removal - Agency shooting	4	1	1	6			
Subtotal Brucellosis Risk Mgt Mortalities	**56**	**858**	**1**	**915**	61%		
Research Removal - APHIS/FWP Quarantine	**0**	**87**	**0**	**87**	6%		
Montana Bison Hunt							
Licensed hunts	8	0	32	40			
Nez Perce treaty hunt	0	0	6	6			
Subtotal Hunting Mortality	**8**	**0**	**38**	**46**	3%		
Traffic Mortality	**15**	**4**	**0**	**19**	1%		
Estimated Natural Mortality & Predation	**0**	**435**	**0**	**435**	29%		
Total Bison Removals & Mortalities	**79**	**1384**	**39**	**1502**			
(1) includes all known mortalities on Highway 191							
(2) includes all other known traffic mortalities inside YNP							
(3) estimated on historic overwinter and predation mortality rates (9% of early-winter population of approximately 4,900 bison)							

ENVIRONMENTAL PERSISTENCE OF BRUCELLA ORGANISMS IN NATURAL ENVIRONMENTS OF THE GREATER YELLOWSTONE AREA - A PRELIMINARY ANALYSIS

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Yellowstone bison, and bison and elk of the southern Greater Yellowstone Areas, are the last remaining reservoirs of bovine brucellosis in the United States. Brucellosis in Yellowstone bison is similar to that of chronically infected cattle (Roffe et al 1999, Rhyan et al 2001). Paired serology and culture tests show about 50% of all seropositive bison, and almost 70% of high titer bison cows, have detectable infection (Roffe et al 1999). Abortion and fetal losses are more common in high titer bison, those who recently seroconverted from negative to positive, and younger primiparious bison. Fluids and tissue from abortions caused by brucellosis contain billions of *Brucella* organisms/gram of tissue and occur primarily in the 3rd trimester. Environmental contamination is caused by aborted material, with the expelled fetus and tissues, feces, fluids contacting soil and vegetation leaving recoverable quantities of living *B. abortus*. Few studies have determined how prevalent these contaminated sites are in the Greater Yellowstone Area and how long these tissues or sites remain contaminated with B abortus in a natural unmanaged landscape.

An important factor in the transmission of brucellosis is the ability of *B. abortus* to survive on agriculturally managed environments (Kuzdas and Morse 1954). Survival in bovine fetuses is

reported to be 135 days in winter when covered with leaves (Cotton, 1919), greater than 2 months in a cool environment (Merck Manual 8th edition), and 180 days in a fetus covered in manure (Nielson and Duncan 1990). Most of the research into the persistence of *B. abortus* in different media (soil, urine, etc.) has been limited in scope and in habitats not typical of the Greater Yellowstone Area. In a recent study, Cook et al., (2002) used *B. abortus* Strain RB51 as a surrogate for field strain and found that in Laramie, Wyoming, the organism survived on the bottom surface of a bovine fetus an average of 60.5 days when the fetus was inoculated in February, 39.5 days in March, 8.8 days in April, 2.8 days in May, and 4.7 days in June. Survival times were much less on the top surface of the fetus ranging from an average of 17.1 days in February to 0.3 days in June. Extremes of survival were 62 days on the underside of a fetus placed in February, 50 days in March, and 18, 4 and 9 days in April, May and June, respectively. In addition, Cook et al, (2004) examined the length of time that a fetus remained in the environment in northwestern Wyoming before it was scavenged. He found that on the National Elk Refuge and Grand Teton National Park most fetuses disappeared within 69.5 hours and the longest period of time until disappearance was 168 hours.

The purpose of this RB51 persistence/fetal disappearance study was to replicate and enhance the work of Cook et al (2002 and 2004) at two sites adjacent to YNP. These two sites, West Yellowstone, MT, near the west entrance to YNP and Corwin Springs, MT, near the north entrance to the Park, are the areas where bison frequently migrate in winter and where cattle are placed on summer pasture. The two sites are somewhat environmentally different with the west site receiving more snowfall and colder temperatures than the north site. In addition, we completed a separate study on the epidemiology of brucellosis in bison by following radio marked bison from 1996-2002. During this study we conducted field investigations at each birth or abortion site that could be found for the purpose of determining prevalence of environmental contamination in naturally occurring birth or abortion events and the persistence of contamination at these sites. In this paper we present the basic persistence data acquired during these various studies to advance our understanding of the risk for transmission associated with these events.

Materials and Methods - Brucella Persistence Study

In a pilot study conducted from February–June, 2001, 16 bovine fetuses were deployed to each of the two study sites in 4 separate sets placed into the field in February, March, April and mid-May. The study areas were north of Yellowstone National Park (YNP) near Corwin Springs inside a protected private property that is surrounded by game proof fence and west of YNP in a fenced and restricted access property used for garbage disposal and recycling. These sites were selected because access could be restricted and biosecurity measures could be implemented by force if necessary.

To emulate a naturally infected fetus each was double bagged and 750 ml. of RB51 solution, containing at least a billion cfu/ml, was poured over the fetus. At both study sites, each fetus was placed in right lateral recumbancy in a large wire dog kennel to protect them from scavengers and provide biosecurity. Each fetus was placed on a 3-4 cm bed of medium coarse gravel.

Eight wire dog kennels at each site were partially covered with shade cloth and eight were uncovered in full sun. Ultraviolet testing at NREL showed that the shade cloth we applied screened 75-80% of the UV radiation.

In addition, two fetuses were placed in wire cages, one shaded and one not, so that temperature probes could be placed and/or sutured in place at the top surface of fetus, bottom surface, in the abdomen, and suspended in the air approximately 15 cm above the gravel bed to record ambient temperature. Temperature probes were connected to data Campbell data-loggers powered with 12-volt marine batteries. Also at each site, UVB radiometers were installed and connected to the Campbell data loggers to measure and record ultraviolet radiation at 12-minute intervals. Data loggers were downloaded weekly.

Immediately following deployment of the fetuses, specimens were collected from each fetus. Specimens consisted of one-cm square biopsies of skin from the top and bottom surfaces of each fetus, and swabs of the abdomen taken through the plastic fistula. Specimens were collected twice weekly from all fetuses at each study area. In 2001 we considered a fetus

negative after only 2 negative results from all three sample sites of each carcass. In 2002 and 2003 we did not consider a fetus negative until 4 consecutive negative results were obtained at which time the fetus was collected and incinerated.

Materials and Methods - Disappearance of Bovine Fetuses

In conjunction with the persistence study described above we conducted a study to determine the length of time a bovine fetus might persist on landscapes within or adjacent to Yellowstone National Park before being scavenged or decomposed. Prior to implementing a large-scale research effort we designed a pilot project in 2001 to evaluate potential research techniques. Two study areas were chosen along the northern boundary western boundaries of YNP within the Greater Yellowstone Area of Montana. In 2001, we acquired permission from the National Park Service and from two landowners living and ranching adjacent to Yellowstone National Park. We were unable to implement this study on US National Forest lands until an environmental assessment was completed due to the sensitive nature of this area of the GYA. In 2002 and 2003 we adjusted our study areas based on results from the initial study and following the completion of an environmental assessment that evaluated impacts associated with placing bovine carcasses in grizzly bear and gray wolf habitat.

In the pilot study we placed 16 cattle fetuses on a study area inside YNP and a complimentary study area on private lands in the northern and western area boundaries of Yellowstone National Park. A motion sensing trap transmitter (ATS) was attached to each rear leg of each fetus to determine when a fetus has been disturbed.

Field teams checked stations several times each week and telemetry sweeps were made routinely. When conducting field checks technicians remained distant from the fetus and used field binoculars to directly observe if the fetus was disturbed. The fetus was not approached until evidence of disturbance or telemetry indicated scavenging activity.

Following the published record of decision by the USFS, permits were acquired in 2002 to work on U.S. National Forest substantially expanding the study area. Furthermore, based on results from the pilot study we chose to redirect efforts to areas outside of Yellowstone National Park where disease transmission concerns are directly relevant. We discontinued using camera's to monitor sites because of the impact of flash devices on scavengers visiting our stations. In addition, the study design was modified to randomly place 4 fetuses each week to more accurately emulate the natural deposition of aborted fetuses on the landscape. In each border study area the suitable winter landscape for bison was predicted by stratifying the study area by open and forested habitats and elevation based upon examining telemetry data from radio-marked bison monitored in 1996-2000. Bison fetuses were placed only within the expected habitat and elevational zone where bison are expected to winter.

Materials and Methods - Brucella Infection at Naturally Occurring Bison Birth or Abortion Sites

Potential birth or abortion sites were located during a cooperative brucellosis epidemiology study conducted from 1996-2001 and interagency field investigations designed to monitor serologically negative pregnant bison in and adjacent to Yellowstone National Park in 2002. From 1996-2001 bison were radio instrumented by field immobilization following techniques identified in Aune et al (1998) and Roffe et al (1998). Each immobilized bison was palpated to determine pregnancy and implanted with a vaginal transmitter if pregnant (Bowman and Jacobson 1998). In 2002, bison were captured during annual field operations under the interagency management plan. Bison were placed into a squeeze and fitted with a radio collar and vaginal implant after an initial screening test was negative for brucellosis. Vaginal implants were motion sensitive and when expelled emitted an increased pulse rate than when remaining in the vagina of a moving bison. These radio-instrumented bison were followed intensively and observed routinely throughout the late winter and early spring period by ground and aerial telemetry searches to locate sites of potential birthing activity (Carstensen et al 2003).

Upon the expulsion of the vaginal-implant device in a marked bison a field investigation was conducted. In addition, during routine field operations there were chance opportunities to

observe unmarked bison calving or aborting as well as encounter aborted fetuses or placental tissue naturally expelled by unmarked bison.

At each site the detailed location of tissue, fluids or fetus were mapped and each micro-site feature was marked with large spike nails. If a fetus or tissue were associated with a site they were carefully collected, bagged, labeled, and frozen. Soil and vegetation were swabbed and then carefully collected beneath each of these birth products and a sample was placed in whirl-pak bags. Within the birth site area samples of soil, vegetation, feces or fluids were collected.

Preliminary Results - Persistence of RB51

In 2001, 4 groups of 16 cattle fetuses soaked in RB51were set out in February, March, April, and mid-May. In 2002 and 2003 bison fetuses became available so were used in the study. With one exception (2002 February) there was no apparent difference in the RB51 survival curves from 2001 samples than in the 2002 and 2003, despite switching to bison fetuses and applying more rigorous standard to declare a fetus negative (4 negatives versus 2 negatives). The average number of days that RB51 survived on tissues at the top and inside the abdomen was typically much lower than for the bottom for all years and months (Table 1). The maximum number of days RB51 survived was 81 days for fetuses set out in February the lowest in samples collected from fetuses placed out in middle of May (21 days). RB51 placed out in all sessions from February through mid-may did not survive beyond June 15 for all three years and both study sites.

Month of deployment	On Top	On Bottom	On swab
Feb	67	77	81
Mar	49	77	63
April	42	69	44
May	21	24	25

Table 1. The maximum number of days that RB51 was detected in fetal tissue by month and location, 2001-2003.

Preliminary Results - Disappearance of Bovine Fetuses

In 2001, 94 bovine fetuses were placed into the field in the Gardiner and West Yellowstone areas. Approximately half of the fetuses were placed inside the borders of Yellowstone National Park while the other half were placed on private lands outside those borders on the northern and western boundaries. There was a significant difference in the mean days until the carcass disappeared between sites within Yellowstone Park (7.5 days) and those outside of Yellowstone Park (13.0 days) ($F=10.10$, $P=0.002$).

Motion sensitive cameras monitored half of the sites where bovine fetuses were deployed. Fetuses disappeared more rapidly at sites without cameras (10.7 days) than those with cameras (17.1 days). It appears that the night flash intimidated some scavengers and may have hindered removal of the fetus from camera-monitored sites. Eleven different scavenger species were photographed scavenging on the fetuses during 2001. Based upon track evidence at least 12 species or groups (like hawks) were scavenging upon these carcasses. In addition, 5 species (elk, bison, jack-rabbits, mule deer, antelope, and Canada geese) investigated the fetuses and several (elk and bison) interacted with the fetus by nudging and contacting the fetus. Most of the scavenging by mammals was during evening or at night while birds scavenged during the daylight hours.

In 2002 and 2003, adjustments were made based upon information obtained from the pilot study in 2001. We discontinued placing carcasses inside YNP as this area is not relevant to the question of temporal separation between cattle and bison. We also adjusted the time relationship for deploying fetuses to more accurately emulate natural abortion events by placing them out 4 per week in a random fashion.

Where fetuses were placed geographically influenced the outcome. Fetuses were placed only outside of YNP and were randomly distributed on a larger landscape, which was made available to the study. The mean number of days until fetuses disappeared at 204 sites outside of YNP was 18.23 days and ranged from 1-78 (S.D.=20.1). There was a subtle but no statistical difference in the median days to fetal disappearance between study areas on the northern (18 days) and western boundary (12 days) (P=0.06). Two outlier points when fetal tissues gradually decomposed rather than scavenged primarily created most of this difference. Animals scavenged all fetuses on the western boundary.

When a fetus was deployed had little effect on the observed outcome. There was no difference in the medians for the months of March (13.5 days), April (13.5 days) and May (14.0 days). Although there was some visible variation between years there was no statistical difference in the median days to disappearance by the years 2001 (20.5 days), 2002 (13.0 days) and 2003 (10.5 days).

Fetuses placed in the field were often transported to one or more locations for scavenging and/or caching. Many (106/204) fetuses moved at least 100 feet from the original deployment site. The maximum distance moved was 2 miles when a fox carried a fetus out on to the ice covering Hebgen Lake to consume it.

Preliminary Results - Brucella Infection at Naturally Occurring Bison Birth or Abortion Sites

Field investigations were conducted at 152 sites with potential to be a birth or abortion site of bison. Approximately half were located with the aid of vaginal implants while the other half were located by chance encounters (Table 2). The greater proportion of sites visited demonstrated some evidence of a birth or abortion event.

Table 2. Characteristics of 152 potential birth or abortion sites investigated, 1996-2002.

Implant	Chance	Birth	Ejection	Marked	Unmarked	Pos. (Marked Bison)	Neg. (Marked Bison)
75	77	96	56	88	64	33	55
49.3%	50.7%	63.2%	36.8%	57.9%	42.1%	37.5%	62.5%

Fourteen of 152 (9.2%) birth sites investigated and sampled were positive for *B. abortus* biovar-1. Two of 56 ejections sites (3.6%) and 12 of 96 birth or abortion sites (12.5%) were culture positive. An aborted fetus was located on 6 of the12 positive birth-sites. Tissues, soil or vegetation were all found to harbor *Brucella* for at least some time period.Persistence was determined through multiple sample efforts for 9 of the14 positive sites investigated. The remaining five sites were available to be sampled only one time for various reasons including heavy snow, flooding or trampling by bison. The bacteria persisted on the April sites (N=6) from 10-43 days but remained viable for only 7-26 days for May sites (N=3).

Preliminary Conclusions

Using RB51 as a surrogate for field strain we found that *Brucella* can persist on fetal tissue exposed to natural conditions in the GYA. RB51 persisted longer on the bottom of fetuses sampled in all sessions deployed from February to mid-may. The length of time RB51 persisted in unperturbed fetal tissues decreased from February through May. None of the RB51 laced fetuses in this study were culture positive after June 15. Scavenging resulted in the rapid removal of most fetuses and fetuses were scavenged more quickly inside YNP than outside. Fetuses were typically scavenged within 40 days; however, some fetuses were not scavenged and naturally decomposed. Soil, vegetation, and tissue at birth or abortion sites naturally infected with field strain *B. abortus* remain infected for up to 43 days in April and 26 days in May. Although sample size is small bacterial persistence of field strain *B. abortus* at birth-sites mimic data from the RB51 persistence study.

In conclusion, preliminary data from these studies indicate that after May 15, the bison haze-back date prescribed in the Interagency Bison Management Plan, natural environmental conditions leading to bacterial degradation and animal scavenging, conspire to kill *Brucella* and remove potentially infected fetal tissue from the environment by June 15.

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