

**Enhancing
Brucellosis Vaccines,
& Vaccine Delivery,
and Surveillance Diagnostics
for Elk and Bison in the
Greater Yellowstone Area**

UNITED STATES ANIMAL HEALTH ASSOCIATION



United States Animal Health Association

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James Roberts, as cited by Leslie A. Dierauf, VMD

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Enhancing Brucellosis Vaccines, Vaccine Delivery, and Surveillance Diagnostics for Elk and Bison in the Greater Yellowstone Area

A technical report by the
United States Animal Health Association
Special Committee on Brucellosis in the Greater Yellowstone Area

August, 2006

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UNITED STATES ANIMAL HEALTH ASSOCIATION

**8100 Three Chopt Road, Suite 203
P.O. Box K227
RICHMOND, VIRGINIA 23288**

November 7, 2004

To: Members of the USAHA Special Committee on Brucellosis in the Greater
Yellowstone Area (GYA)

Thank you for attending the discussion/planning meeting at our 108th Annual Meeting on October 25, 2004 in Greensboro, North Carolina and your willingness to serve and provide leadership on this important issue. As a follow-up to that meeting, on the last day of the Annual Meeting I announced the formation of this special committee with President-Elect Bret Marsh serving as chair.

As you know, my first charge to this special committee is to plan a working symposium to address the research needs for new and improved vaccines, delivery systems and diagnostics for use in bison and elk, and the cost for that research. This is the first charge of this special committee. There may very well be others.

Dr. Marsh and I have had several discussions on how to proceed. In the near term, we would like to first hold a conference call to begin the planning process, to be followed by a face-to-face meeting at some centrally located site. He is working on the details for those meetings.

Again, thank you for your leadership. I look forward to working with you on this important issue.

A handwritten signature in black ink that reads "Richard D. Willer". The signature is fluid and cursive, with a long horizontal stroke at the end.

Richard D. Willer, DVM
USAHA President



United States Department of Agriculture
Office of the Secretary
Washington, D.C. 20250



United States Department of the Interior
Office of the Secretary
Washington, D.C. 20240

JUN 13 2005

Dr. Bret Marsh
Indiana State Board of Health
800 Beachway Dr., Suite 50
Indianapolis, IN 46224

Dear Dr. ^{BRET}Marsh:

Thank you for agreeing to participate in the *Greater Yellowstone Area Brucellosis Vaccine, Diagnostics, and Delivery Systems Workshop* in Laramie, Wyoming, from August 16 – 18, 2005. This workshop is being convened by a special committee of the United States Animal Health Association (USAHA) chaired by USAHA President-elect, Dr. Bret Marsh, with primary funding provided by the United States Departments of Agriculture and the Interior. Your experience and expertise in these areas will help make this workshop successful.

The Greater Yellowstone Area (GYA) is one of the largest intact temperate zone ecosystems on Earth, includes approximately 28,000 square miles in Montana, Idaho, and Wyoming and is home to approximately 100,000 elk, 5,000 bison, and widespread livestock on private and public lands. Brucellosis (*Brucella Abortus*) was first detected amongst wildlife in the GYA among Yellowstone bison in 1917. Although we can never be certain, infection of GYA elk and bison with brucellosis is assumed to have been through initial co-mingling with infected livestock, and subsequently has been maintained through co-mingling of wildlife. Arising from the highly successful national brucellosis eradication program among domestic livestock and captive wildlife, GYA elk and bison are now recognized as the last large reservoir of *Brucella abortus* in the United States.

All elk and bison populations in the GYA are variably, but chronically, infected with brucellosis. Northern GYA elk exhibit low seroprevalence levels (1-3 %) whereas southern GYA elk associated with feed grounds can exhibit variable, but much higher, seroprevalence levels (15-60 %). In recent years, bison in the Jackson Hole, Wyoming, area have been utilizing the National Elk Refuge feed ground and exhibit high seroprevalence levels (70-80 %), while there is long-term trend of 40-50 % seroprevalence among Yellowstone National Park bison. At different times and under different jurisdictions, brucellosis management strategies have included combinations of capture/test/slaughter, vaccination, surveillance, and spatial/temporal separation from livestock (hazing and shooting). At present, brucellosis management programs are based on serologic tests that identify bison and elk that, at a minimum, have been exposed at some

unknown level to *Brucella abortus*. There are no currently available efficient or effective surveillance diagnostics on live animals to separate those that have been only exposed to *Brucella abortus* versus those that are actually infected.

There are very important gaps in the technical capacity to conduct highly effective elk and bison brucellosis vaccination and surveillance. Specifically, there are widely acknowledged capacity gaps regarding vaccine safety and efficacy, delivery system safety and efficacy, and diagnostics. It is important to note, however, that vaccination is only one tool that can be used in a brucellosis elimination strategy and is unlikely to be successful if relied on alone. To be effective in an overall brucellosis elimination effort, vaccination will likely need to be utilized in conjunction with other management techniques. There are also significant knowledge gaps in the use of some of those additional techniques in wildlife.

The USAHA Special Committee has organized this workshop to bring together key individuals from federal, state, academic and private sectors to formulate a Strategic Action Plan to enhance brucellosis vaccines, vaccine delivery, and surveillance diagnostics for bison and elk in the GYA. Working with the USAHA Special Committee, the Ruckelshaus Institute of Environment and Natural Resources at the University of Wyoming has developed an exciting workshop agenda and is providing meeting facilitation and assisting with the arrangements. You will receive separate correspondence from the Ruckelshaus Institute regarding workshop arrangements.

We heartily encourage your participation and contributions toward identifying solutions to these key technologies that will contribute to the elimination of brucellosis from the GYA while maintaining thriving wild and free-ranging wildlife populations.

Sincerely,



Bill Hawks
Under Secretary
Marketing and Regulatory Programs



 For Craig Manson
Assistant Secretary
for Fish and Wildlife and Parks



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June 28, 2006

Rick Willer, DVM
Immediate Past President, United States Animal Health Association
1688 West Adams St.
Phoenix, AZ 85007

Dear Dr. Willer,

At the 2004 United States Animal Health Association (USAHA) Annual Meeting in Greensboro, North Carolina, you appointed a USAHA Special Committee on Brucellosis in the Greater Yellowstone Area (GYA) and requested that I serve as the Chair of this Special Committee. The Special Committee was charged to plan and implement a working symposium to address the research needs for new and improved vaccines, vaccine delivery systems and diagnostics for brucellosis in bison and elk, and the cost for that research.

For over a century, USAHA's mission has been to protect animal and public health by serving as a national forum for communication and coordination concerning disease management and animal health, serving as a clearinghouse for new information and methods for policy and programs development, and developing solutions for animal health issues. This report continues upon our tradition of science-based consensus building.

Through primary funding assistance by the US Department of Agriculture and the US Department of Interior, the Special Committee worked with the University of Wyoming Haub School and Ruckelshaus Institute of Environment and Natural Resources to hold the working symposium August 16-18, 2005 at the Laramie campus of the University of Wyoming. The preliminary results of the working symposium were reported at USAHA's 109th Annual Meeting in Hershey, Pennsylvania.

On behalf of the Special Committee, it is my honor to present to you the Final Report from the working symposium entitled "Enhancing Brucellosis Vaccines, Vaccine Delivery, and Surveillance Diagnostics for Elk and Bison in the Greater Yellowstone Area." This comprehensive technical report includes state-of-the-art authoritative assessments from the 43 invited technical experts and 15 members of the Special Committee who participated in the working symposium; and is an unparalleled assessment of vaccine and surveillance opportunities for meeting the challenges of brucellosis in the GYA.

Sincerely,

Bret D. Marsh, DVM
President

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Executive Summary

The United States Animal Health Association (USAHA) Special Committee on Brucellosis in the Greater Yellowstone Area (GYA) sponsored a working symposium on August 16-18, 2005 at the University of Wyoming in Laramie to identify the research needs and costs for the development of vaccines, vaccine delivery systems and diagnostics to address brucellosis (*Brucella abortus*) in bison and elk. The University of Wyoming's Helga Otto Haub School and William D. Ruckelshaus Institute of Environment and Natural Resources was selected to serve as the facilitator of the working symposium utilizing primary funding provided by the United States Departments of the Interior and Agriculture.

One hundred and twenty-five participants, Special Committee members and observers successfully identified several areas for further research to address the challenges of brucellosis in the GYA. The highest priority research needs in each of the three areas of discussion follow.

Vaccine Development:

- Empirical or applied research – There is a need to establish a protocol to rapidly screen new vaccine candidates for efficacy in bison and elk. Currently available brucellosis vaccines must be immediately utilized in preclinical efficacy studies and field-testing in bison and elk.

- Discovery or basic research – There is also a need to expand our knowledge of pathogenesis, protective antigens, and immunologic responses to *B. abortus* in bison and elk. If vaccines evaluated with empirical approaches prove unacceptable, then the knowledge gained through the basic research approach might offer alternative vaccine solutions. Several key areas were identified within this approach:
 - Reproducible disease models for bison and elk
 - Surrogates of protective immunity
 - Host specific immunologic responses
 - Antigen discovery
 - Continual adjuvant/formulation/delivery optimization
 - Novel *B. abortus* genetically-engineered vaccines
 - Durability of immunogenicity

The costs associated with the research needs in vaccine development depended upon the research approach. It was estimated that to develop and license a promising vaccine would cost approximately \$10M. To conduct further research on existing vaccines would cost approximately \$400,000-\$500,000 per study per species over a period of 1-2 years.

Vaccine Delivery Systems:

- The highest priority research areas for delivery systems are:
 - Oral baits – methods that require an animal to ingest the vaccine
 - Biocompatible bullets – the vaccine is delivered directly to the subcutaneous tissues or deeper
 - Natural forage dispersed vaccine – utilizes a dispersed antigen externally applied to natural forage
 - Transdermal – any method that delivers a vaccine by direct absorption through or into epidermal tissues
- The selection of these delivery methodologies for use in bison and elk in the GYA is based upon the following assumptions:
 - A delivery system is heavily dependent on the vaccine type
 - Existing systems need additional development
 - Due to the complexity of brucellosis in the GYA, multiple platforms for delivery may be necessary
 - Social and ecological considerations are a must to gain public acceptance
 - A system must be cost effective with the ability to access large numbers of animals
 - Appropriate funding must be available to adequately develop the delivery system
 - Field validation trials must be conducted.

Diagnostics

- There is a need to validate the existing diagnostic methods that are applied to wildlife. Although originally developed for cattle, many of the current diagnostic tools have been extrapolated for use in wildlife. The World Organization for Animal Health (OIE) standards for validation could be used as a guide.
- There is a need to establish a clearinghouse for sharing information that also identifies a process for sharing reagents, contains a master database, and maintains a repository for well-characterized diagnostic materials.

The following priorities were categorized by the time necessary to reasonably accomplish the goal.

Short-term (1-2 years)

- Meta-analysis of the current data through the incorporation of existing publications as well as unpublished findings to determine the existing base of knowledge on diagnostic tests for brucellosis.
- Standardization of the Polymerase Chain Reaction (PCR)

Intermediate term (2-5 years)

- Biomarkers
- Vaccine Markers
- Matrix Antibody/Antigen

Long term (5-10 Years)

- Rapid diagnostic tests (genomics and proteomics)



The costs associated with accomplishing the highest priority research needs in the development of diagnostic tools were estimated at \$28-\$52M. Host genomics were considered separately, and the estimated cost of this research would add \$30M to the overall cost.

Crosscutting Issues

- There is a need to establish a collaboration consortium to facilitate and oversee the brucellosis research efforts on bison and elk. This oversight group will assist in identifying and procuring funds, prioritizing research, and coordinating multidisciplinary research teams.
- There is also a need to evaluate how to facilitate this research since brucellosis is designated as a select agent (i.e., listed by the Center for Disease Control and Prevention as having the potential to pose a severe threat to public health and safety). Currently, this designation makes it very difficult to conduct the research necessary to address the brucellosis challenge.
- Additionally, there is a need to identify the facility needs to conduct the necessary research. Currently, facilities for brucellosis research that can house several large animals for long periods of time in a Bio-Safety Level 3 Ag environment do not exist in the United States.

The working symposium was a significant step in the long journey to address the brucellosis challenge in the GYA. It was the first time that technical experts from around the world were assembled with the specific task of addressing the vaccine, vaccine delivery and diagnostic challenges of bison and elk in the GYA. The participants willingly shared their thoughts and ideas, and their efforts have established a course of action.



Introduction

Bret Marsh

Indiana State Veterinarian, Indianapolis, Indiana, Chair of the Special Committee on Brucellosis in the Greater Yellowstone Area

Rick Willer

Arizona State Veterinarian, Phoenix, Arizona, President of the United States Animal Health Association

Background

The Greater Yellowstone Area (GYA) is one of the largest intact temperate zone ecosystems and includes approximately 28,000 square miles in Montana, Idaho, and Wyoming. Besides these state lands, the GYA encompasses two national parks, portions of six national forests, three national wildlife refuges, Bureau of Land Management holdings, and private and tribal lands. The GYA is also home to the largest wild elk and bison populations in the United States. Approximately 125,000 elk occupy the GYA across 25 separate exclusive or trans-boundary elk management jurisdictions. Agencies manage elk and their habitat resources through complex interagency cooperation. Elk hunting occurs in all elk management jurisdictions except Yellowstone National Park (YNP). There are also 23 elk feedgrounds in northwest Wyoming (the National Elk Refuge and 22 Wyoming state operations) that can support approximately 25,000 elk, depending on winter severity. In 2005, approximately 5,000 bison occupied



Bret Marsh, Chair, USAHA Special Committee on Brucellosis in the Greater Yellowstone Area, welcoming the attendees.

the GYA in and adjacent to YNP (4,200) and Jackson Hole, Wyoming (800). Bison are managed under several bison management jurisdictions. Bison hunting presently occurs only in select national forest areas in Wyoming. Most bison in Jackson Hole utilize the National Elk Refuge feedground during winter and are not susceptible to hunting.

Brucellosis was first detected among wildlife in the GYA in bison in 1917. Although we can never be certain, infection of GYA bison with brucellosis was assumed to have occurred initially by commingling with infected

livestock, and subsequently has been maintained through commingling among bison and elk. Following the highly successful national brucellosis eradication program among domestic livestock and captive wildlife, GYA elk and bison are now recognized as the last large reservoir of *Brucella abortus* in the United States. The regional and national importance of brucellosis in wildlife has been recognized by the responsible agencies since the early 20th century. Since then, these agencies have implemented a variety of livestock, wildlife, and disease management strategies that have been extensively reviewed by the National Research Council (NRC), General Accounting Office (GAO), and the Greater Yellowstone Interagency Brucellosis Committee (GYIBC).

Elk and bison throughout the GYA are chronically infected with brucellosis to various degrees. Northern GYA elk exhibit low seroprevalence levels (1-3%) whereas southern GYA elk associated with feedgrounds can exhibit much higher seroprevalence levels (15-60%). Bison in Jackson Hole, which have recently begun utilizing the National Elk Refuge feedground, exhibit high seroprevalence levels (70-80%), whereas there is a long-term trend of lower (40-50%) seroprevalence among bison not on feedgrounds. Taking abundance, distribution, and management strategies into account, an admittedly coarse calculation suggests that there may be nearly 12,500 brucellosis-seropositive elk and 2,500 brucellosis-seropositive bison in the GYA. However, these calculations are uncertain because of the difficulty of diagnosis. At present, brucellosis management programs are based on serologic tests that identify bison and elk, which at a minimum, have been exposed at some unknown level to *B. abortus*. There are no efficient or effective surveillance diagnostics on live animals to separate those that have been only exposed to brucellosis versus those that are actually infected.



At different times and under different jurisdictions, brucellosis management strategies have included combinations of capture/test/slaughter, vaccination, surveillance, and spatial/temporal separation from livestock (hazing and shooting). The NRC stated that “Vaccination in bison and elk is one part of an overall strategy that could be used to control or eliminate *B. abortus* in the GYA, but much research is needed before current vaccines can be judged adequate for use in those species.” (NRC 1998).¹ A recent GYIBC review of brucellosis management options described the existing technical and management capabilities to eradicate brucellosis from the GYA and came to the same general conclusions as the NRC (GYIBC 2002).² There are very important gaps in the technical capability to conduct highly effective elk and bison brucellosis vaccination and surveillance. Specifically, there are acknowledged gaps regarding vaccine safety and efficacy, delivery system safety and efficacy, and surveillance diagnostics.



Working symposium attendees.

The Working Symposium

At the 2004 United States Animal Health Association (USAHA) annual meeting in Greensboro, North Carolina, the 2005 president, Dr. Rick Willer, appointed a Special Committee on Brucellosis in the GYA. The first charge of the Special Committee was to plan and implement a working symposium to address the research needs for new and improved vaccines, vaccine delivery systems, and diagnostics for brucellosis in bison and elk, and the costs for that research. The results of the working symposium were reported back to the USAHA president at the 109th annual meeting in Hershey, Pennsylvania.

Primary funding for the working symposium came from the U.S. Departments of Interior and Agriculture. The Special Committee utilized the services of the University of Wyoming’s Helga Otto Haub School and William D. Ruckelshaus Institute of Environment and Natural Resources to organize and facilitate a stakeholder working symposium. The working sympo-

¹ National Research Council. 1998. Brucellosis in the Greater Yellowstone Area. National Academy Press, Washington, DC. 186 pp.

² GYIBC. 2002. Brucellosis in Elk and Bison in the Greater Yellowstone Area. Terry Kreeger (ed.), Wyoming Game and Fish Department, Cheyenne, WY. 171 pp.

sium brought together key individuals from federal, state, academic, and private sectors to formulate a Strategic Action Plan for an “Initiative to Enhance Brucellosis Vaccines, Vaccine Delivery, and Surveillance Diagnostics for Bison and Elk in the Greater Yellowstone Area.” The intent of the Strategic Action Plan was to describe the overall framework and level of support required to: 1) develop and test enhanced vaccines for safety and efficacy in applications to bison and elk; 2) develop and test safe and effective vaccine delivery options; and 3) improve live-animal diagnostic capabilities in distinguishing seropositive from infected animals.

Recognizing that effective vaccines, delivery systems, and diagnostic tools are part of an overall strategy to eliminate brucellosis from the GYA, the Strategic Action Plan may reference other tools, such as management techniques, which may contribute to the overall goal of elimination of brucellosis.

A total of 43 participants from the United States and three foreign countries (Canada, Russia, and New Zealand) attended the working symposium following an exhaustive selection process by the Special Committee (Appendix A). Participants were selected based upon their scientific expertise in the areas of vaccine development, delivery methods, and diagnostics. Each participant was placed in one of three groups tasked to address these three areas. Periodic plenary sessions provided an opportunity for all workshop participants, as well as the nearly 60 observers, to hear and comment on the deliberations of each group.

On the first morning of the working symposium, a virtual tour of the GYA was presented to familiarize the participants with the complexity of the brucellosis issue in the area. Fourteen of the participants had toured the GYA preceding the working symposium as well (Appendix B). Presentations describing the management constraints in developing products for use in wildlife (Appendix C), delivery methods (Appendix D), and diagnostics (Appendix E) were given to improve the participants’ overall understanding of the issues. To emphasize the interrelationship among multiple jurisdictional agencies in the GYA, presentations were given by Bob Moon (National Park Service, U.S. Department of the Interior), Valerie Ragan (Animal and Plant Inspection Service, U.S. Department of Agriculture), Keith Aune (representing state wildlife agencies), and Jim Logan (representing state animal health agencies). Presentations by three participants from Russia, Alexander Denisov, Roman Borovick, and Konstantin Salmakov, provided an international perspective on brucellosis issues and research (Appendices F, G, and H).

On the final afternoon of the working symposium, a facilitated public information exchange was held to inform stakeholders of the working symposium’s initial findings and to invite comments and questions.

The Report

The following report, prepared by the Special Committee, contains the results of the working symposium held at the University of Wyoming on August 16-18, 2005. This report identifies possible vaccines, delivery systems, and diagnostics for use in bison and elk and prioritizes research needs and their associated costs. Drafts of this report were reviewed by all of the invited participants.





Report of the Vaccine Working Group

Chairs:

Steve Olsen

U. S. Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, Iowa

Phil Mamer

Idaho Department of Fish and Game, Caldwell, Idaho

Section Participants:

Gerry Andrews, Lorne Babiuk, Randy Berrier, Wendy Brown, Lynette Corbeil, Alexander Denisov, Phil Elzer, Mark Estes, D.L. Lodmell, Julie McMurray, Scott McVey, Russ Midgough, Charles Mihaliak, Frank Milward, Peter Nara, David Pascual, Konstantin Salmakov, Ron Schultz, Gerhardt Schurig, LeeAnn Thomas, Ramesh Vemulapalli

Background

The persistence of brucellosis in bison and elk, caused by infection with *B. abortus*, poses a continued risk for transmission to domestic livestock and humans. Although vaccination is probably the most attractive control measure, vaccination alone will probably not eradicate brucellosis from free-ranging bison and elk. Also, some scientists question whether a vaccination program is even capable of reducing brucellosis prevalence in bison and elk. Limited data





Vaccine working group at the working symposium.

for bison suggested that the *B. abortus* strain 19 vaccine was not efficacious when administered as a calfhooed vaccine and it also caused a high percentage of abortions. The efficacy of the *B. abortus* strain RB51 vaccine in bison remains in dispute. One research group reported that RB51 was efficacious as a calfhooed vaccine whereas another group disputed this. There does appear to be consensus that the strain RB51 vaccine is safe as a bison calfhooed vaccine and that it does not induce serologic re-

sponses, which would interfere with identification of bison infected with field strains of *B. abortus*. Data also suggested that route of delivery may influence immunologic responses of bison to vaccination.

There also appears to be consensus by scientists that the strain RB51 vaccine is not efficacious in elk, but that the strain 19 vaccine induces limited protection. Data suggested that elk developed strong humoral responses, but poor cellular immune responses, after vaccination with strains 19 or RB51. Strong cellular immune responses are the best correlates of protective immunity against brucellosis known at this time. The lack of cellular immune responses after vaccination may explain why elk are not protected by current *B. abortus* vaccines. Current knowledge suggests the possibility that different vaccines may be required in order to protect both bison and elk against brucellosis.

Vaccine Working Group

Participants charged with reviewing and writing the report in the vaccine working group were derived from academic, government, and private industry with expertise in the areas of vaccine development and production. The overall mission of the vaccine working group was to design a sustainable, innovative, basic and applied vaccine research and discovery program for application towards the eradication of infection and disease caused by *B. abortus* in bison and elk. The working group recognizes that the application of vaccines to these types of dynamic problems is not an independent solution for eradication. However, vaccines can be an effective disease mitigating tool that, when used in conjunction with other known practices such as test and slaughter or habitat manipulation, could lead to the eventual eradication of *B. abortus* from free-ranging elk and bison in the GYA.



Other management strategies may include, but are not limited to, phasing out feed grounds with transition to native winter range, habitat restoration, mandatory transition zone vaccination of cattle, and perhaps removal of *B. abortus*-positive animals. Other issues that the committee discussed included vaccine delivery methods and routes (particularly if multiple doses of vaccine are required), vaccine influence on protective responses, and environmental issues. All of these issues will likely have some influence on the generation of protective responses.

The working group focused on utilizing consortium-building strategies and established vaccine development programs used by industry and vaccine scientists. The working group hopes that the *B. abortus* vaccine development program outlined here will also provide a template for future needs addressing other wildlife diseases certain to challenge modern society. Areas were identified where there was either a complete lack of knowledge, an underdeveloped or fragmented knowledge base, or significant gaps for developing an effective vaccine for this application. Based on these discussions, the committee identified three main areas of emphasis for addressing vaccine research needs:

1. Interrelated issues which will influence research progress, implementation, and efficiency.
2. Empirical approaches to rapidly screen new vaccine candidates for efficacy in bison and elk.
3. Discovery or basic research approaches to expand knowledge on pathogenesis, protective antigens, and immunologic responses to *B. abortus* in bison and elk to facilitate development of new second and third generation vaccines.

Interrelated Issues

The working group identified important programmatic issues at the local, state, national, international, and/or regulatory levels in an effort to facilitate brucellosis vaccine development for bison and elk while maximizing productivity and efficiency. In this area, the committee identified the need for: 1) a coordination team; 2) identification of funding sources; 3) addressing regulatory policies which hinder *B. abortus* vaccine research; and 4) sufficient biocontainment facilities to conduct the identified research.

The committee suggested that a group of professionals representing national animal disease groups (e.g., USAHA) and state and federal agencies be formed to oversee efforts in the area of bison and elk brucellosis research. This oversight group would assist in identifying and procuring funding, in prioritizing research needs, and in coordinating multidisciplinary or consortium research teams that would integrate vaccine, diagnostic, and delivery arenas. In regards to vaccine research, the oversight committee would disseminate progress reports from both the empirical and discovery approaches to all interested parties such that research approaches in vaccines, vaccine delivery, and diagnostics are integrated and coordinated.

The working symposium committee identified funding as a major concern for progress in bison and elk vaccine research. Costs for individual studies would be high due to the pur-



Working symposium attendees share information.

chase price of sufficient animals for experiments, husbandry expenses, research supplies, and meeting government licensure regulations, which specify appropriate biocontainment for research with *B. abortus*. In addition to direct funding from state and federal special purpose legislation, other sources of funding could include commodity groups, private foundations, and ongoing programs in state and federal agencies. In addition, the biologics industry could contribute to this effort by providing discovery and de-

velopment expertise. In some cases, this could include limited direct support. It is not expected that a vaccine for a very limited population of elk and bison would represent a commercial market. Manufacturing of released vaccine would require specialized and perhaps subsidized support.

Regulatory issues as specified by the Select Agent Act and the Agricultural Bioterrorism Act of 2002 were also identified by the working group as being significant roadblocks for progress in brucellosis vaccine research. The ability to share *B. abortus* strains is impaired by these regulations, although there is a regulatory process whereby vaccine strains can be declared exempt from the Select Agent Act on an individual basis. Because these regulations also specify biocontainment requirements for *B. abortus* vaccine research, they directly impact which animal and laboratory facilities are available for conducting research addressing bison and elk brucellosis. Currently, biocontainment facilities which could conduct efficacy experiments in bison and elk are extremely limited and cannot accommodate large numbers of animals. This lack of facilities is having a detrimental impact on research progress and this will be a critical limiting factor for some time. The working group discussed the fact that licensure of outside facilities is being considered, although it was emphasized this was not assured at this time. Facilities in other countries may be useful to conduct brucellosis vaccine research, especially in the near future.

Empirical or Applied Research Approach

As a short-term approach (1-5 years), the working group suggested a strategy of utilizing currently available brucellosis vaccines (worldwide) that are in later stages of research development for immediate preclinical efficacy studies and field testing in bison and elk. Evaluations



would use definitions of efficacy and safety as adopted by the GYIBC in May, 1998. As part of this empirical approach, vaccine evaluation would be conducted such that data would be valid for use in obtaining licensure from the Animal and Plant Health Inspection Service – Center for Veterinary Biologics (APHIS-CVB). It is expected that available antigen candidates will be evaluated, but this evaluation would include broader investigations to look at formulations and routes of delivery. Development of promising candidates would continue in a streamlined approach directed toward conditional licensure and eventual full licensure. Issues regarding relationship to master seed, manufacturing processes, safety trials, and field trials would need to be addressed in the design of experiments such that licensure is facilitated for promising candidates.

Briefly, the working group suggested that current data on existing new vaccines be reviewed and the best candidate(s) tested in bison and/or elk. Immunized and control animals would be challenged using current standard dose and routes and vaccine-induced protection determined. This approach should be integrated with the discovery approach to maximize productivity and to obtain data that would address key basic research needs such as identification of correlates of protective immunity.

Discovery or Basic Research Approach

The working symposium committee identified the need for a basic research component to be simultaneously initiated along with the applied research approach. The strategy for this is to explore novel approaches, establish basic science practices which yield incremental discoveries, and develop information which will facilitate advances in diagnostics and vaccine development. In the event that all vaccines evaluated under the empirical approach prove unacceptable, knowledge gained through a basic research approach should offer alternative vaccines that might be successful in bison and elk. Several key areas were identified within this approach:

- Reproducible disease models for bison and elk
- Correlates of protective immunity
- Host-specific immunologic responses
- Antigen discovery
- Continual adjuvant/formulation/delivery optimization
- Novel *B. abortus* genetically-engineered vaccines
- Durability of immunogenicity.

Reproducible Disease Models for Bison and Elk

The standard methodology for evaluation of vaccine efficacy in ruminants against *B. abortus* is based on work conducted more than 60 years ago. Efficacy is based on resistance to infec-

tion or abortion after intraconjunctival delivery of 1×10^7 CFU (colony-forming units) of *B. abortus* strain 2308 during midgestation. The working group felt that research methodology should be standardized so that infection or pathogenesis models give reproducible results. Use of standard methodology may not obtain information quickly because efficacy experiments in bison can take approximately three years for completion if calfhoo d vaccination is being evaluated.

Surrogates of Protective Immunity

The working group suggested that alternative surrogates (experimental animal or tissue models) be identified that correlate with protective immunity so that efficacy may be evaluated more quickly. This would, in turn, facilitate a possible conditional registration by the U.S. Department of Agriculture (USDA) to allow use of the vaccine. It should be emphasized that this approach requires a model in which the host species is protected against brucellosis, a requirement which the working symposium committee was not certain existed for elk. Possibilities for positive or negative correlates of protection include, but are not limited to, bacterial killing, bacterial adherence, phagocytosis, tissue colonization, cellular differentiation, cytokine transcription, acute-phase response, or antibody-based assays. Measurements of innate immunity using microbial pathogen-associated molecular patterns (PAMPS) may also correlate to protection. Due to a lack of species-specific immunologic reagents in bison and elk, this objective may require development and sharing of reagents.

Host-specific Immunologic Responses

Current knowledge suggests that there are phenotypic differences in bison and elk compared to cattle regarding their response to infection by virulent *B. abortus* strains or by vaccination with *B. abortus* strains 19 or RB51. This is particularly noted with elk where vaccination failed to develop robust cell-mediated responses and subsequently demonstrated poor protection. To develop a vaccine with the greatest efficacy, it will be necessary to understand how bison and elk immune systems respond to standardized test antigens, current/new vaccines, or infection with virulent strains of *B. abortus*. A basic understanding of host/pathogen interactions and regulation in response to *B. abortus* infection or vaccination may identify targets or strategies for modulation of immune responses in a manner that increases protection. In a similar manner, knowledge of genes which facilitate persistence in the host, or assist in evading defenses, may be valuable in developing genetically-engineered new vaccines.

Antigen Discovery

Development of new, more efficacious vaccines is impaired by the fact that the genes or proteins of *B. abortus* which mediate protective immunity are not well defined in any species, let alone bison and elk. Data have identified some brucellosis antigens which play a role in protective immunity, such as Cu/Zn superoxide dismutase or L7/L12 ribosomal protein. Current knowledge does not support the conclusion that currently identified antigens are the only genes which mediate protection. The currently identified protective antigens have primarily



been generated using laboratory animal models of *B. abortus*; antigens which induce protective immunity in bison and elk may be markedly different. Evaluation of early host responses may identify *B. abortus* antigens which invoke non-protective or ineffectual immunologic responses. Use of genomics, proteonomics, microarrays, or computer modeling to identify antigens which stimulate B- and T-cell responses are approaches that may be beneficial. Protective antigens might also be identified by using molecular techniques to express recombinant proteins with immunologic screening in the host species of interest.



Konstantin Salmakov, All Russian Veterinary Institute, discusses his research as UW student Evguenia Arzamazova assists with translation.

Because antigen processing and expression are critical components for induction of protective immunity, prospective protective antigens may need to be expressed and delivered using different platforms in order to induce protective immunity in bison and elk. Identification of genes which mediate protective immunity combined with proper delivery may allow immunologic responses to be focused, which may enhance efficacy in the host species of interest.

Adjuvant/formulation/delivery Optimization

Multiple methods for immunopotentialization of vaccines are currently available including adjuvants, CpG sequences, microencapsulation techniques, and others. Alternative types of vaccines such as DNA vaccines, inactivation technologies, and plant or baculovirus vehicles are also available. Some of these techniques have already been successfully used to develop efficacious vaccines for other pathogens. These techniques or procedures may prove beneficial in developing more efficacious brucellosis vaccines for bison and elk. It should be emphasized that studies to identify the optimal antigen concentration, vaccine formulation, or delivery regimen will have to be conducted to maximize protective immunity induced by a promising candidate. Regardless of how efficacious a new vaccine candidate appears, it will be useless if it cannot be manufactured in sufficient amounts.

B. abortus Genetically-engineered Vaccines

Current vaccines are primarily live bacterial vaccines because live bacteria have proven most efficient at inducing long-term protection against brucellosis. However, live *B. abortus* vaccines pose zoonotic risks for humans, can induce abortions in pregnant animals, and require

refrigeration to maintain viability. Targeted genetic engineering of live *B. abortus* strains may initially be the most viable approach for development of new brucellosis vaccines. Targets for genetic modification might include genes involved in pathogenesis, genes involved in protective immunity, or genes which misdirect the immunologic responses toward non-protective responses.

Durability of Immunogenicity

Because the lifespan of bison can exceed 20 years and elk 10 years, durability of any viable brucellosis vaccine candidate will need to be determined. Knowledge of the duration of immunity of any vaccine considered for use in the GYA will be critical in the development of an effective vaccination program for control of brucellosis in elk and bison.

Summary

The importance of the current brucellosis infection in bison and elk in the GYA should not be underestimated. The current impact on Wyoming and other regional cattle industries is economically and politically significant. Clearly, efforts to eradicate *B. abortus* from bison and elk are justified. An important part of the effort should include both basic immunological research and target vaccine development. The nature of this problem requires that potential, easily-achieved strategies be investigated. However, it is necessary to continue basic research because it is questionable if any of the currently available vaccine candidates will provide solid immunity, especially in elk. Progress of both direct development and basic research may be synergistic and may serve to identify an effective vaccine in the most time-efficient manner. In any case, vaccination is only one tool potentially available for management of brucellosis in bison or elk. The efforts to control this disease will only be successful through integration of multiple management tools including habitat management, improved diagnostics, delivery of efficacious vaccines, and population management.





Report of the Vaccine Delivery Working Group

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Background

The use of brucellosis vaccines to immunize elk and bison in the GYA has been debated for several decades. Although there remains much disagreement whether vaccination will eliminate brucellosis, the prevailing view is that vaccination may help to reduce the incidence of brucellosis as long as the vaccine is safe. While vaccines have routinely been used to immunize livestock, there are many obstacles and challenges when attempting to apply current vaccines and vaccine delivery systems to free-ranging wildlife.





Alexander Denisov, Research Center for Toxicology and Hygienic Regulation of Biopreparations, Ministry of Health of the Russian Federation, testing the biobullet delivery system.

Vaccines are rarely used to manage wildlife disease. Two novel programs employing large-scale vaccination of wild animals in the U.S. are the Wyoming elk brucellosis vaccination program and the oral rabies vaccine (ORV) program in the eastern United States and Texas. In the Wyoming elk vaccine program, strain 19 vaccine is remotely delivered, at relatively short ranges, to elk calves via a biodegradable casing containing freeze-dried vaccine. This program has successfully delivered vaccines to elk by attracting animals to a winter feedline to mitigate natural

avoidance behavior and overcome ballistic delivery limitations. Oral rabies vaccine programs are currently expanding, but illustrate the many challenges relative to environmental regulations, program costs, and target specificity. Neither of these programs has successfully eliminated a disease. The ORV program has demonstrated some measure of disease control, but the efficacy of elk brucellosis vaccination for disease control is debatable. Controlled experiments suggested that the elk vaccine may be 25-30% efficacious.

There are two fundamental components required for successful brucellosis vaccination in wild elk and bison: 1) an efficacious and safe vaccine against *B. abortus*; and 2) an effective and safe method of delivering the vaccine. Although the development of safe brucellosis vaccines for wildlife seems imminent, the challenges of reliably delivering effective vaccines to free-ranging wildlife remain considerable.

Most of the existing and current research on vaccine delivery has focused on parenteral (administered by some means other than the digestive tract) and ballistic delivery techniques. These techniques are often limited by the necessity of capturing or containing free-ranging wildlife or by the close approach distance necessary to remotely deliver vaccines. The research conducted to date has emphasized extending the range of remote delivery systems, improving ballistic characteristics of the biobullet delivery system, increasing payload of biocompatible bullets, and improving overall effectiveness of remote vaccine delivery. Research on oral delivery has only recently been started and has focused on: 1) developing attractive feeds or baits that can bind with vaccines; 2) determining species-specific preferences for feeds or baits; and 3) designing approaches for delivering oral vaccines to elk and bison.



The focal topic and goal of the vaccine delivery working group was to define a variety of existing or potential vaccine delivery methods that could be used in free-ranging wildlife within the GYA. Furthermore, the working group was tasked with identifying priority research needs and evaluating the cost, potential timeline, and possible barriers to conducting this research.

Delivery Systems Workgroup “Brainstorming”

The workgroup created a comprehensive list of potential vaccine delivery methods and identified what factors affected these methods. The working group stratified vaccine delivery systems into five major categories based on the route of delivery (Table 1). Under each major category, an expanded list of specific methodologies was developed through a “brainstorming” exercise (Table 2).

As the discussions expanded and became diffuse, the working group established some discussion guidelines to help focus the group. The group chose to exclude discussion of vaccine delivery methods for cattle. The working group then developed a general list of ancillary constraints that affected the delivery of vaccines to free-ranging wildlife and identified some of the general barriers that would affect all vaccine delivery systems (Table 3).

Prioritization

The expanded list of potential vaccine delivery methods was prioritized (high, medium, or low priority) by consensus to further discussion and evaluation of the methods (Table 4). Due to time constraints and in an effort to work efficiently, the working group avoided any detailed analysis and discussion of low priority methods. The prioritization tended to class delivery systems that are currently available or in the process of development as high priority, while high-risk approaches having high potential returns tended to be classed as medium priorities. Following the identification of high research priorities, the workgroup identified major research questions, timelines, costs, and additional specific barriers associated with each (Table 5). For the medium priority methods, the group identified the appropriate research questions to be considered but did not address cost, timelines, and specific barriers (Table 6).

Conclusions

- Vaccine delivery systems are heavily dependent upon vaccine type. It is essential to understand the biochemical and molecular properties of the vaccine(s) before an efficient vaccine delivery system can be fully developed.
- There are several existing delivery systems in place, but these need additional development to become effective vaccine delivery tools for the GYA.
- Given the great complexity of brucellosis management in the GYA, multiple platforms for delivering vaccines are needed. It is highly unlikely that one system will work for all areas, in all circumstances, and in both species.



- Social and ecological considerations will be very important in gaining public acceptance for vaccination and the various approaches to delivering vaccines.
- Effective delivery must be cost effective and able to reach a large number of animals in a large landscape. The logistical challenges will be great.
- Funding will be a major barrier preventing the research and eventual use of vaccines and the development of appropriate and effective delivery systems.
- Field validation trials should be conducted to evaluate effectiveness of vaccine delivery before widespread application of vaccination programs in the GYA.

Table 1. Five categories of delivery systems to be considered based on the route of administration.

Route of Administration	Method
1. Oral	Any method that depends upon an animal ingesting a vaccine.
2. Injectable	Any method by which the vaccine is directly delivered to subcutaneous or deeper tissue.
3. Transdermal	Any method that delivers a vaccine by direct absorption through or in epidermal tissues.
4. Biologically vectored	Any method that introduces a vaccine by use of living organisms associated with target host.
5. Non-oral mucosal	Any means of delivering vaccine across a mucosal surface but excluding ingestion.



Table 2. Potential vaccine delivery methods.

Delivery Approach	Potential Methods
Oral	<ul style="list-style-type: none"> • Applying vaccine to baits • Spiking natural or artificial water sources • Incorporating vaccines into salt attractants or within natural salt licks • Natural forage enhancement (using a dispersed antigen externally applied to natural forage) • Engineered recombinant forage (using genetic engineering to incorporate antigen into forage) • Other recombinant forms • Transgenic approaches • Innovative encapsulation of vaccines (packaging live bacteria, DNA, or antigens into smaller capsules for delivery) • Application to surfaces (applying antigens to the surface of an attractant that animals may contact such as a fetus)
Injectable	<ul style="list-style-type: none"> • Dart delivery (degradable or recoverable) • Trap, vaccinate and release • Biocompatible bullets (such as the biobullet) • Delivery of a depot • Application to antlers/horns (to take advantage of fighting)
Transdermal	<ul style="list-style-type: none"> • Ballistic (remote delivery of vaccine in a salve, paste or patch that contacts the skin surface) • Contact (delivery of vaccine through natural rubbing and/or other grooming behaviors)
Biologically vectored	<ul style="list-style-type: none"> • Using biting arthropods to inject vaccine • Using other viruses/bacteria to delivery vaccine by infection • Phage • Nematodes/other parasites that can deliver vaccine by infection
Mucosal	<ul style="list-style-type: none"> • Aerogenic (aerosol) delivery to mucosal surfaces of the nose and throat. • Bioengineered venereal disease • Ocular delivery (aerosols into the membranes of the eye; e.g., dead antigen with a polymer in aerosol form used to treat pink eye) • Rectal suppository

Table 3. Ancillary factors affecting delivery methods and some general barriers affecting all delivery approaches.

Issue	Factors
Some con- straining fac- tors are heavily dependent on the vaccine type being con- sidered	<ul style="list-style-type: none"> • Achieving sustained release (inside GI tract or in an injected depot) • Biomarkers are needed to evaluate vaccine delivery • Concerns of single versus multiple dosing (long-acting or requiring a booster) • Available adjuvant systems • The horizontal/vertical amplification of vaccines (vaccine system that delivers to select members of cohort and transmitted to other members of the cohort or offspring) • Vaccine package duration/stability and storage/shelf life • Vaccine dose regulation (age and gender specificity and product stability) • Vaccine clearance from target and/or non-target animals • Target/non-target species and specific biosafety issues • Environmental impacts and ecological concerns are important considerations <ul style="list-style-type: none"> * Ecological consequences may often be unforeseen <ul style="list-style-type: none"> - Trophic level impacts may occur. - Natural selection processes and population genetics may be altered in unforeseen ways - There may be effects on population demographics by altered fertility or reproduction - Delivery systems may unnaturally concentrate animals - Intrusive methods (aircraft or high human disturbance) may disperse animals and affect seasonal movements * Biosafety: there may be adverse effects on non-target and/or target species including humans (hunters) that consume wildlife * Persistence of a vaccine or delivery system products in the natural environment is often unknown
Some con- straining considerations are not as de- pendent on the vaccine type	<ul style="list-style-type: none"> • There will be species-specific responses to vaccine and delivery methods • Product availability could be limiting (who can produce, when can it be delivered, and how much can be manufactured) • Active versus passive delivery approaches (differences in vaccine methods that are delivered by humans to individual animals versus methods that self-vaccinate animals by some innovative means) • Aerial delivery logistics (there are significant challenges in managing aerosols when they are delivered in natural landscapes) • Remote vaccination devices: mechanical devices that are remotely activated by sensing or by contact with the animal are difficult to regulate



Issue	Factors
Potential barriers to research and field applications	<ul style="list-style-type: none"> • Biosafety research needs will be significant for most new vaccines • Regulations and policy (various jurisdictions will apply) • Facility availability, especially with respect to “BL3” (biosafety level 3) facilities • Costs (funding for basic and applied research) • Limited effective range for many of the existing delivery methods • Formulation of adjuvants and/or delivery packages for some methods • Social acceptance of vaccination programs and specific delivery methods

Table 4. Prioritization of vaccine delivery methods.

Priority	Delivery Method
High	<ul style="list-style-type: none"> • Oral delivery • Baits, salt or feed • Biocompatible bullets <ul style="list-style-type: none"> * depot injectable * biobullets • Natural forage dispersed vaccine • Transdermal-surface application of vaccine
Medium	<ul style="list-style-type: none"> • Dart systems • Arthropods as a biological vector • Phage as a biological vector • Viruses/bacteria/nematodes as biological vectors • Aerogenic or aerosol • Recombinant forage
Low	<ul style="list-style-type: none"> • Rectal suppositories • Parasites other than nematodes as vectors • Antlers/horns • Trap, vaccinate, release (TVR; already available) • Spiked water sources • Other recombinant forms • Bioengineered venereal disease • Oral delivery from surfaces • Transgenic



Table 5. Research needs, cost, timelines and barriers for high priority vaccine delivery systems for elk and bison in the GYA.

Research Need	Costs	Timeline	Questions	Specific Barriers
Basic research	Low	Long-term	<ol style="list-style-type: none"> 1. Can we develop a multiple platform approach specific to various circumstances? 2. What kind of biomarker is needed and how do we incorporate it into delivery systems? 3. What is the effect of multiple vaccinations? 4. Can we develop a sustained release approach for many of the proposed systems? 5. Field validation experiments are necessary for all approaches. 	<ol style="list-style-type: none"> 1. Multiple platforms create additional complexity. 2. Environmental compliance for field validation experiments.
Oral baits	Low-medium	Short-term	<ol style="list-style-type: none"> 1. Can we develop baits that are attractive to elk and bison? 2. Will the baits be compatible with vaccines? 3. How do we stabilize the bait vaccine so that it can remain stable in the environment and reach the target tissues? 4. What kind of bait vaccine package is needed? 5. How do we make an oral bait vaccine package with sustained release and proper dosing properties? 	<ol style="list-style-type: none"> 1. Uncontrolled access to baits or attractants by multiple species. 2. Significant probability of delivering a vaccine to non-target species.



Research Need	Costs	Timeline	Questions	Specific Barriers
Biocompatible bullets	Medium	Short-term	<ol style="list-style-type: none"> 1. How do we develop stable ballistic characteristics? 2. What are the effective working ranges? 3. Can we develop ballistic delivery with adequate payloads? 4. What mortality or morbidity is associated with ballistic delivery? 5. What are the behavior responses of animals and how can we mitigate them? 	<ol style="list-style-type: none"> 1. The physics of ballistic systems can be limiting in application and can produce harm to animals. 2. Animal behaviors may be significantly modified with unforeseen consequences.
Natural forage dispersed vaccine	Medium-high cost depending upon formulation	Technology development is short-term; formulation development is long-term	<ol style="list-style-type: none"> 1. Can we develop methods for applying candidate vaccines to forage and forage products? 2. How do we regulate dosing? 3. What is the stability of the vaccine in the environment? 4. How do we stabilize the bait vaccine? Questions of stability so that we can reach the target tissues and stability of vaccine in the environment. 5. What is the effect on forage palatability and acceptance by elk and bison? 	<ol style="list-style-type: none"> 1. Uncontrolled use of a vaccine in natural environments. 2. Significant probability of delivering a vaccine to non-target species.
Transdermal	High	Technology and formulation will be long-term	<ol style="list-style-type: none"> 1. What is the effective penetration necessary for a ballistic transdermal delivery? 2. Are there alternatives to ballistic delivery of a transdermal vaccine? 	<ol style="list-style-type: none"> 1. A great deal of uncertainty surrounds this novel approach until the basic research is completed.



Table 6. Research questions associated with medium priority delivery approaches.

Delivery Approach	Research Questions
Darts	<ol style="list-style-type: none"> 1. Can we develop a sodium bicarbonate slow injection system? 2. Can we develop biodegradable darts that are ballistically competent? 3. Improved retention devices are needed for dart systems.
Arthropods as Vectors	<ol style="list-style-type: none"> 1. Do you sterilize biological vectors to prevent reproduction and if so, how? 2. Develop the necessary husbandry protocols for mass production of the arthropod. 3. Can we develop protocols to insure target specificity when dealing with a free-living organism? 4. Will the vectored vaccine produce the desired immune expressions?
Virus/Bacteria/Nematodes/Phage as Vectors	<ol style="list-style-type: none"> 1. What vectors can be used? 2. Can vectored antigen express and protect (achieve the desired immune presentation)? 3. Can we manage dose? 4. Is containment possible? 5. Genetic stability of the vector? 6. Need research on the basic gut microflora with respect to introduction of vectors.
Aerogenic (Aerosols)	<ol style="list-style-type: none"> 1. How do we create an effective aerosol with <i>B. abortus</i>? 2. Specificity of delivery? 3. Containment and targeting of vaccine. 4. How do you administer to a whole population? 5. Environmental persistence of vaccine?
Recombinant Forage	<ol style="list-style-type: none"> 1. What do we combine antigen with? 2. Dosing/concentration issues. 3. Specificity of delivery? 4. Effects of chronic intake on immunity?





Report of the Diagnostics Working Group

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Background

The process required to develop better diagnostic tools is complicated and may be influenced by advances in vaccine development. The suggestions provided in this section are based upon what is currently known and what is possible with adequate funding. Developments in other areas, such as vaccines, may influence diagnostic needs. For example, if vaccines with biomarkers are developed, diagnostic tests capable of detecting the biomarker will also need to be



developed. Development of diagnostic assays will be greatly enhanced by initially conducting more fundamental research (e.g., a better understanding of host immune systems or mapping host genomes). Therefore, the overall development of improved diagnostics will be an evolving process that requires considerable and open communication between many researchers working in the brucellosis arena. Further, much of what can be learned through this process may be applicable to research in other diseases in these species and may result in improved investigations of wildlife diseases in general.

Needs

It is important to note that there were several concerns and needs that should be addressed if diagnostic development is to move forward. First, the participants placed a very high priority on the validation of existing diagnostic methods that are applied to wildlife. Many of the current diagnostic methods have been developed for cattle and extrapolated for use in wildlife. Standardized validation methods need to be developed after which current diagnostic tools need to be validated for wildlife. Several participants recommended utilizing the OIE (World Organization for Animal Health) standards for validation as a guide. Because so many agencies are testing various animals in different scenarios, the goal of the validation exercise is to provide data-supported recommendations for the best methods to use. Validation generally requires large numbers of animals, which could be difficult for studying elk and bison. Participants also expressed the importance of including several laboratories in the validation process to assure widespread applicability and repeatability.

The need for a true “gold standard” for the diagnosis of brucellosis was also discussed. Although culture has always been considered such a standard for the diagnosis of brucellosis, the inability to successfully and consistently culture from known infected animals has resulted in an imperfect “gold-standard.”

The current select agent designation of *B. abortus* causes substantial challenges to the ability to work with the bacteria in captive bison or elk because it is difficult to confine these animals in BL3 indoor facilities. Since it is unlikely that the Centers for Disease Control (CDC) and the USDA will change the status of this bacterium or defer status for select research projects, alternatives and methods to allow controlled outdoor research need to be developed and approved.

Facilities are also a limiting factor in brucellosis research in livestock and wildlife. Animals need to be contained, often for long periods of time (e.g., up to ten years), to evaluate new diagnostic tests and to infect them for vaccine testing. Few facilities are available that can accommodate large animals for long periods and none are expected to be developed in the near future. In consideration of this situation, the USDA has developed a checklist necessary to work with *B. abortus* in outside pens. The document is due for release for comment soon.

During the course of the discussion, it became apparent that there is a great need for centralization of information. The participants felt this could be best met via the establishment



of a clearinghouse for the coordination of research and diagnostic projects. Included in this clearinghouse would be the establishment of 1) a process to share necessary reagents, 2) a master database, and 3) a repository for well-characterized diagnostic materials. Participants felt that a consortium should be formed to provide a framework to accomplish these necessary tasks. The consortium would consist of partners working in the brucellosis arena (i.e., universities, private industry, government diagnostic laboratories and agencies).



Dennis Slate, USDA-APHIS, and Rick Willer, 2005 USAHA President, at the working symposium.

The Question

To begin the process of obtaining ideas, participants were asked the following question:

What are the most important research needs for improving live-animal diagnostic capabilities to distinguish naturally-infected animals from vaccinated animals?

The participants were asked to answer the question without constraint, including funding. In addition, the co-chairs suggested that tests for wildlife should have 1) high sensitivity and specificity, 2) ease of development, 3) rapid results, and 4) ease of use in the field (i.e., not limited to a laboratory). There was considerable discussion of a test that could measure the infectivity of an animal. Some participants felt that any seropositive animal should be considered infectious regardless of actual status. This is important for managing wild herds since infected animals often are intermittently infectious. Participants subsequently developed 23 suggestions for potential diagnostic techniques (Table 7).

Prioritization

The participants then prioritized their suggestions based on 1) how quickly the projects can be completed, 2) use in live animals, 3) chance of success, and 4) funding requirements. Participants also estimated the time required to complete research on these topics. The timelines were divided into short term, intermediate, and long term. The participants noted that the suggestions designated as top priority met the above criteria. However, other suggested projects were also important. Several of the projects may also depend upon results from other working symposium groups.



Meta-analysis of current data and standardization of current polymerization chain reaction (PCR) tests were high priorities that could be accomplished in the least amount of time at relatively low expense. Metadata analysis would involve combining and statistically analyzing data from published and unpublished sources to more fully understand the existing base of knowledge on brucellosis diagnostic tests. Dr. Klaus Nielsen provided examples of brucellosis test data currently available (Appendix 3). Research topics, such as “Biomarkers and Vaccine Markers,” are examples of areas of interest that could be shared by the vaccine and diagnostic sections. Clearly, developments in one arena could benefit the progress in the other.

The development of rapid tests was also designated as a high priority. This project was selected as a top priority, even though it was long-term, because rapid tests can provide an overall benefit to understanding and managing diseases in elk and bison. The participants felt that determining host and bacterial genomes would take time (thus the long-term status), but once accomplished, rapid tests could then be developed more quickly. Expense is a major hurdle because determining the host genomes would cost millions, which should be considered in terms of the overall cost-benefit ratio. However, once completed, the applicability of this information could be very broad.

Improving culture methods for *B. abortus* was also discussed. Some participants felt that advancements in technology could be applied to the improvement of culture techniques. Cultures could also benefit advances in both vaccine development and diagnostics by confirming results. Cultures should be a part of the repository (tissue bank) suggested as a source of bona fide samples used in research and diagnostics.

Diagnostic Techniques

A number of possible diagnostic techniques were discussed. Time limitations prevented in-depth discussions on specifics, so possible diagnostic techniques were grouped under broad categories (Table 7). These categories are: 1) Immunological - techniques related to antigen/antibody characteristics and interactions; 2) Genomics - techniques related to genetic makeup and/or gene response or manipulation; 3) Proteomics - techniques related to proteins, particularly their structures and functions; 4) Chemical – techniques related to the detection of certain chemicals produced by the body or organism; 5) Epidemiology – techniques related to the study of the disease itself and its behavior; and 6) Others.

Funding

Funding estimates were developed for various diagnostic research projects. This was difficult because funding depends upon many unknown factors. Notably, determination of host genomes would be very expensive (estimated at \$30 million). Adding this cost to the budget would greatly increase funding requirements. Considering the top priorities and facility needs, as well as several other suggested projects, the participants estimated a budget from \$28-52 million. Host genomics should be presented as a separate case.



Table 7. Summary of suggested diagnostic techniques. Highest priority items are in bold face. E = elk, B = bison, O = organism.

Area	Platform	Short-term 1-2 years	Intermediate 2-5 years	Long-term 5-10 years	Priority 1 = high 2 = medium 3 = low
Immunological	Rapid Tests (genomics and proteomics)			X	1
	Matrix Antibody/Antigen		X (E and B)		1
	Vaccine Marker (vaccine dependent)		X		1
	Latency			X (E and B)	3
	Protein Micro-array		X (E and B)		2
	Raman (antibody)	X (E and B)			3
	Phage Reagent/Aptamer		X		3
Genomics	Standardization of PCR (and improving bacterial isolation technology)	X			1
	Up/Down Regulation of Genes		X		3
	Sequencing (bacterial and genome of host)			X (E, B, O)	2
	Genetic Response (susceptibility)			X (E and B)	3
	ID of Biomarkers		X		2
Proteomics	T-Cell Biomarkers		X (E and B)		1
	2-D Gels et al.			X	3
	In Vivo Express Protein Gel			X (E and B)	3
Chemical	Raman	X (O)			3
	Urine/Breath	X (E and B)			3
Epidemiology	Meta-Analysis	X			1
	Locating <i>B. abortus</i> on Landscape			X	3
	Integrate with Behavioral Patterns			X	3
	Environmental Detection Method	X			
Other	Remote sensing			X	3
	Improved culture			X	3



Appendix A

Working Symposium Participants, Special Committee Members, and Staff

Participants

VACCINE BREAKOUT GROUP

Gerry Andrews, Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming

Lorne Babiuk, Canadian Vaccine Institute, University of Saskatchewan, Saskatoon, Saskatchewan

Randy Berrier, Colorado Serum Company, Denver, Colorado

Wendy Brown, Department of Immunology, Microbiology & Pathology, Washington State University, Pullman, Washington

Lynette Corbeil, University of California Medical Center, San Diego, California

Alexander Denisov, Ministry of Health of the Russian Federation, Moscow, Russia

Phil Elzer, Department of Veterinary Science, Louisiana State University, Baton Rouge, Louisiana

Mark Estes, Sealy Center for Vaccine Development, University of Texas, Austin, Texas

D.L. Lodmell, Rocky Mountain Laboratories, Hamilton, Montana

Julie McMurry, EpiVax, Inc., Providence, Rhode Island

Scott McVey, Pfizer Animal Health, Lincoln, Nebraska

Russ Middaugh, Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas

Charles Mihaliak, Dow AgroSciences, Indianapolis, Indiana

Frank Milward, Merial, Ltd., Athens, Georgia

Peter Nara, Biological Mimetics, Inc., Frederick, Maryland

David Pascual, Veterinary Molecular Biology, Montana State University, Bozeman, Montana

Konstantin Salmakov, All Russian Veterinary Institute, Kazan, Tatarstan, Russia

Ron Schultz, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin

Gerhardt Schurig, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia

LeeAnn Thomas, U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Riverdale, Maryland

Ramesh Vemulapalli, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana



DELIVERY BREAKOUT GROUP

Keith Amass, Safe Capture, Inc., Mount Horeb, Wisconsin

Roman Borovick, Ministry of Health of the Russian Federation, Moscow, Russia

Bryce Buddle, Wallaceville Animal Research Centre, Upper Hut, New Zealand

Paul Cross, U.S. Geological Survey Northern Rockies Science Center, Bozeman, Montana

Allison Ficht, College of Medicine, Texas A&M University, College Station, Texas

Dave Grainger, Colorado State University, Fort Collins, Colorado

Rick Hansen, SolidTech Animal Health, Inc., Newcastle, Oklahoma

Lowell Miller, National Wildlife Research Center, Fort Collins, Colorado

Charles Rupprecht, Centers for Disease Control, Dept. of Health and Human Services, Atlanta, Georgia

Dennis Slate, U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Concord, New Hampshire

Gary Wobeser, Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, Saskatchewan

DIAGNOSTICS BREAKOUT GROUP

Betsy Bricker, National Animal Disease Center, Ames, Iowa

Pat Fitch, Lawrence Livermore National Laboratory, Livermore, California

Emilio Garcia, Lawrence Livermore National Laboratory, Livermore, California

Ted Hadfield, Midwest Research Institute, Palm Bay, Florida

Sharon Hietala, California Animal Health and Food Safety Lab, University of California at Davis, Davis, California

Ken Mills, Wyoming State Veterinary Laboratory, Laramie, Wyoming

Klaus Nielsen, Animal Diseases Research Institute, Canadian Food Inspection Agency, Ottawa, Ontario

Stacy Tessaro, Animal Disease Research Institute, Lethbridge, Alberta

Richard Warren, Battelle Memorial Institute, Columbus, Ohio

Mark Wolcott, U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, Maryland

Wei Ling Yu, Animal Disease Research Institute, Ottawa, Ontario



SPECIAL COMMITTEE

L. Garry Adams, Texas A&M University, College Station, Texas

Keith Aune, Montana Fish, Wildlife & Parks, Bozeman, Montana

John Clifford, U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Washington, DC

Frank Galey, College of Agriculture, University of Wyoming, Laramie, Wyoming

Terry Kreeger, Wyoming Game & Fish Department, Wheatland, Wyoming

Tom Linfield, Montana Department of Livestock, Helena, Montana

Phil Mamer, Idaho Fish & Game Department, Caldwell, Idaho

Bret Marsh, Indiana State Board of Animal Health, Indianapolis, Indiana

Steve Olsen, U.S. Dept. of Agriculture, Agriculture Research Service, Ames, Iowa

Glenn Plumb, National Park Service, Yellowstone National Park, Wyoming

Valerie Ragan, U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Riverdale, Maryland

Jack Rhyan, U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, Colorado

Tom Roffe, U.S. Fish & Wildlife Service, Bozeman, Montana

Rick Willer, Arizona State Veterinarian, Phoenix, Arizona

Scott Wright, U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin

STAFF

University of Wyoming Haub School and Ruckelshaus Institute of Environment and Natural Resources:

Melinda Harm Benson

Harold Bergman

Ann Boelter

Nancy Hoffer

Nicole Korfanta

Aaron Laur

Jill Lovato

Josh Moro

and

Molly Mayo, Meridian Institute, Dillon, Colorado





Appendix B

Field Trip Itinerary and Participants

Pre-Working Symposium Field Trip – August 12-15, 2005

Field Trip Leaders:

Glenn Plumb, Yellowstone National Park

Keith Aune, Montana Department of Fish, Wildlife and Parks

Field Trip Hosts:

Mark Atkinson, Montana Department of Fish, Wildlife and Parks

Neal Anderson, Montana Department of Fish, Wildlife and Parks

Ryan Clarke, Animal and Plant Health Inspection Service

John Treanor, Yellowstone National Park



*Field trip participants at
Bison Quarantine Project.*

Field Trip Participants:

- | | |
|-------------------------|---|
| 1. Roman Borovick | Ministry of Health of the Russian Federation |
| 2. Lynette Corbeil | University of California San Diego Medical Center |
| 3. Alexander Denisov | Ministry of Health of the Russian Federation |
| 4. Pat Fitch | Lawrence Livermore Labs |
| 5. Maria Koller-Jones | Canadian Food Inspection Agency |
| 6. Phil Mamer | Idaho Dept. Fish & Game |
| 7. Russ Middaugh | University of Kansas |
| 8. Charles Mihaliak | Dow AgroScience |
| 9. Peter Nara | Biological Mimetics |
| 10. Charles Rupprecht | Centers for Disease Control |
| 11. Konstantin Salmakov | All Russian Veterinary Institute |
| 12. Dennis Slate | U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service |
| 13. Stacy Tessaro | Canadian Food Inspection Agency |
| 14. Rick Willer | U.S. Animal Health Association |



August 12

- Tour Paradise Valley, Montana with Montana Fish, Wildlife and Parks, [Keith Aune, Kurt Alt and Tom Lemke]
- Tour Bison Quarantine Project with Animal and Plant Health Inspection Service, [Ryan Clarke]
- Tour Gardiner Basin, Stephens Creek, Madison-Firehole with Yellowstone National Park, [Tim Reid, Brian Helms, Glenn Plumb]



Field trip participants in Yellowstone National Park.

- Tour Horse Butte with Montana Department of Livestock, [Tom Linfield and Rob Tierney]
- Tour Grizzly Discovery Center, West Yellowstone, MT

August 13

- Drive through southern YNP enroute to Jackson Hole, Wyoming
- Tour selected areas in Buffalo Valley, Elk Ranch, Kelly with Grand Teton National Park, [Sue Consolo-Murphy and Steve Cain]
- View National Elk Refuge with Fish and Wildlife Service [Steve Brock]
- Visit Wyoming state elk feedground with Wyoming Game and Fish [Ron Dean]

August 14

- Drive from Jackson to Pinedale, WY
- Tour WY elk feed ground and meet with area livestock producers, with Wyoming Game and Fish Department [Scott Werbelow], and Wyoming State Representative [Monte Olsen]

August 15

- Presentation on brucellosis issues in Idaho with Idaho Fish and Game Department [Phil Mamer]
- Drive from Pinedale to Laramie





Appendix C

Overview of Management Constraints to Consider in Developing a Wildlife Vaccine, Delivery System, and Diagnostic Tools.

Leslie A. Dierauf, VMD

U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin

In general terms, field work with wildlife requires creativity and ingenuity along with considerable understanding of the particular species with which you are working. I will focus on some of the technical considerations necessary for working with wild animals as well as other compromising factors such as permits, legal jurisdiction, and political will. It is important to remember that wild animals are free-ranging and, aside from non-natural obstacles such as roads, fences, and canals, they have few boundaries. They exist in the open environment and as such are subject to the whims of the day or season. They do not recognize political boundaries. They are not livestock or pets. They are not domesticated. Generally, they are wary and do not tolerate people. Because they avoid people, they are not easily approachable. They can hurt



people either accidentally (attempting escape) or intentionally (protecting their young). Some would argue that humans share the earth with wildlife; however, experience has taught us that, in some circumstances, humans are guests in the world of wildlife. In the arena of wildlife diseases, the focus is on the effect of the disease on the population rather than the emphasis on the individual animal. For

this reason, disease (as with other population limiting factors) is managed at the population level.

Conducting field research with wildlife requires considerable planning. Whenever possible, experienced people should either be a part of, or be consulted by, the team going into the field. To accomplish goals of any wildlife study, increased time of contact and closeness of contact (hands on) leads to increased risk to both researchers and wildlife alike. Considerable thought needs to occur in order to assure that the work being done is necessary and brings added value or need to what is already known about the particular wildlife species involved. With increased risk comes greater consequence. Problems occur, especially if the wildlife species is rare, endangered, or one with great political interest. Errors in judgment can be fatal to both research and disease management programs.

Knowing the Host

A critical aspect of planning for a field project is understanding the biology of the species involved. From a wildlife disease perspective, the animal is considered the “host” of the disease. Different host species often differ biologically even when they coexist. These hosts have different biological clocks, movement patterns, reproductive seasons, food preferences, and tolerance of human activity. Some can be handled or captured more easily than others. A very important piece of information for understanding the severity of a disease is the frequency of occurrence of the disease in the population. The size of a population of free-ranging wildlife is rarely known accurately. For this reason, notions of frequency of disease are only estimates. Larger species (such as bison and elk) have seasonally directed movement throughout the year. Animal migration can be a good way of gaining access to large numbers of animals over a short time; however, as the animals are “on the move” they may be less easily captured. There is rarely a reliable indication of their health or nutritional status at the time of capture. Unless



it is very obvious, all animals are generally considered healthy if they are behaving normally. Subtle differences (modified immune systems, early stages of disease) are not apparent and are unknown at the time the animals are captured. The capture and handling process may accelerate pre-existing conditions. So the value of the capture and handling of wildlife needs to be considered against the risk.



There is always risk when working with free-ranging wildlife.

Wildlife comes in different shapes and sizes. A project involving field mice has a different level of complexity than a study of cheetah. Generally, larger animals are more powerful and, therefore, potentially more dangerous. Beached whales are not aggressive per se but they can be deadly when they roll over people standing next to them on the beach. Handling or restraining larger animals has its own set of complications, especially if these animals have claws and sharp teeth. Some species cannot lay on their sides any length of time or they will suffocate. Some wildlife species panic when their faces or eyes are covered, while others calm down. Even if chemical restraint is used to subdue an animal and chemical reversal is employed to “bring it back,” there needs to be a plan of what to do and where to be when the animal awakens, because the animal is not usually very “happy” upon awakening.

The timing of handling wild animals is critical. The time required to capture the animal and the weather conditions (e.g., temperature, approaching storm) during the capture need to be considered, as does the actual time you will need to collect your samples. If the study requires many samples using multiple procedures with varying invasiveness, the process needs to be triaged, so that the most important samples are collected first. Manually restrained animals (non-chemical) do not generally tolerate restraint beyond a certain point, so quick decisions need to be made about completing sets of samples originally planned. Chasing animals to capture them is physiologically stressful and can be fatal (capture myopathy; hyperthermia). Times need to be determined beforehand as to when to break off capture attempts if your efforts exceed that timeframe. The experience of capture and handling may affect the immune system through a general stress response, which may compromise vaccination as a result of immune stimulation. The stress effect will abate with time but if the vaccine is a weak immune stimulant, it may not be as effective as desired.

The Disease Agent

It is equally important to understand the biology of the disease agent as it is to understand the biology of the host. There are several important disease agent characteristics to consider when working with free-ranging animals. Field staff need to exercise extreme care if a disease agent is highly infectious and can be transmitted by mechanical means (equipment, clothing, instruments, foot wear, etc.) in addition to direct contact with infected hosts. Staff could be a direct source of spreading disease to uninfected wildlife hosts by poor management of their activities. Actively capturing and handling wild animals infected with zoonotic diseases has an additional level of concern because the disease can infect humans as well. Extreme care is necessary if working with a highly infectious zoonotic disease. Animals can facilitate transmission by throwing feces, projectile vomiting, projectile urination, or defecation. All of these excreta could be highly infectious. Some disease agents are hardy and able to withstand harsh conditions (wide range of temperature extremes, survival in soil, resistance to many conventional disinfectants), while others are fragile (easily degraded by sun or desiccation) and do not survive outside a living host. Decontamination of equipment is more difficult in the field. Assuring the animal's skin is clean prior to vaccine injection or blood collection is not a trivial exercise. Utilizing different instruments for each animal is critical to minimize the possibility of spreading a disease agent among wildlife. Working with "select agents" is very limited in the wild. Some work with select agents can occur if the animals can be housed in containment, but this is also limited by rules and regulations.

The Environment

Climate and seasonal weather conditions are major factors affecting field work. Temperature extremes, particularly in the West, can occur during a single 24-hour period. These can have very different influences on activities and careful planning can avoid such setbacks. In some respects, it may be easier to work in colder weather, as long as it not too extreme. Warm weather gear can be lighter and items that need to be chilled are easier to maintain. Hot weather requires reliable sources to keep samples or vaccines cold. Cold packs, wet ice, and dry ice all become portable means to keep crucial materials from overheating. Hot weather is more dangerous, since it amplifies the heat generated by the animal undergoing capture exertion. When an animal is chemically restrained, even when chase times are limited, some drugs can accelerate metabolism, subsequently raising core body temperature. Thus, steps need to be taken to keep the animal cool during handling.

Local weather, which is sometimes ignored, can be a constraint to handling animals. Large, dangerous storms can build up quickly, especially during very hot weather spells. The associated lightning and strong winds and rain can scatter animals and can be very dangerous for



field staff, especially in remote locations. Higher elevations are subject to sudden snow squalls and abrupt drops in air temperature that can be life threatening. Someone on the team should be aware of the local weather and abort capture efforts before animals or personnel become threatened.

You

An important, but sometimes overlooked, component in the challenge of wildlife disease work in the field is the scientist.

As conditions become more severe and the location more remote, the physical difficulty and associated level of risk increases. If the best time of the year to get to your animals is the middle of winter, then you are forced to wear bulky cold weather gear that impedes your movement and makes it far more difficult to trudge through the snow to conduct procedures. Something as simple as collecting blood can be compromised by cold temperatures. Not only does the investigator need to remove gloves or mittens, but cold temperatures reduce the vacuum in blood collection tubes so that they need to be warmed up before they will work properly. Hot temperatures in the field are dangerous not only for capturing wildlife, but also for the scientist, especially if water is not carried in because of weight constraints in remote locations. The severity of the disease agent can require considerable personal protective clothing that is bulky and awkward, which is made even more so in the field.

Most government scientists are required to have a considerable amount of training and certification before they can conduct field work with wildlife. Proficiency must be demonstrated in the use of firearms, boats, all-terrain vehicles, capture guns, and immobilization drugs. Wilderness first aid and survival training along with flight training are usually required for personnel who will need to fly into remote locations to work. Various state or federal legal permits are often required as well. Government scientists are required to obtain the same permits as university scientists. Special areas, such as national parks, require a substantial permit process that can take months to obtain. Tribes often require permission to enter and work on their lands. Private landowners need to give permission to enter and work on their lands as well. If the disease agent is considered a select agent, special authority needs to be granted to transport and use the agent, and the parent laboratory and staff must be registered in the select agent program.



Field trip participants view map of elk feedgrounds in Wyoming.



Logistics

Even a relatively accessible study site requires planning and logistical considerations. Usually, all of the necessary equipment is transported into the field. Foremost in equipment is adequate first aid and emergency communications equipment. Cell phones are not always the best means of communication because they do not work in remote locations. Trucks can transport several people and lots of

equipment, but they are loud and can get easily stuck in ruts, mud, or sand. So if the vehicle scares the animals, it has reduced value even if it has a greater capacity to transport material. As a compromise, trucks can be used up to a point and then equipment is hand-carried into the work area, but then weight and space become critical issues. Studies that utilize helicopters can transport equipment and staff to a base site and then move out to capture animals and return to the base site to process the animals. Alternatively, the helicopters can bring a processing team to a capture site, drop them off, proceed to another capture site, return for the team, and so forth. Experience dictates that equipment and supplies can fail. Needles bend and sample collection tubes break. This necessitates replacements, which take up limited space and increases weight. There is always the question of how much to bring along because the laboratory is usually miles and hours away.

Some initial processing in the field is often very helpful to provide the best quality samples. This usually requires a power source. Power sources take up space and can add considerable weight. Each power source needs to be reliable. Battery power is compromised in extreme cold temperatures. Gasoline-powered generators are loud and require highly flammable gasoline adding to risk. Even solar systems may not work if the sun doesn't shine.

Teamwork

Larger projects usually work best with a team of people with varying degrees of expertise. The team works best when coordination has occurred before the fact and instructions are given prior to moving to a remote area to insure everyone is capable of their role. The team leader needs to insure the team practices its actions together. Once the team is in the field and hits its rhythm and works smoothly, the results can be remarkable. With more people involved and paying attention to the situation, accidents are less likely to occur. The leader needs to focus the team, so that everyone understands the mission, risks, and possible consequences or changes, since circumstances can and do change abruptly. Just as everything is carried in, it needs to be carried out. Wastes are not left in the field. Contaminated instruments and cloth-



ing are isolated (usually bagged) and transported back to the laboratory for decontamination and disposal. Waste material of any kind should not be buried in the field because it can be dug up and transported by scavengers. In general, more heads and hands are better than fewer, and this applies to wildlife studies in the field and laboratory.

Collaboration

Science has advanced to the point where none of us can work alone. There are not enough resources, be they people, funds, supplies, samples or resources, to go around. We must work together to leverage our resources and come up with solutions. The time has passed for competition among or between scientists.

My recommendations are:

1. Take a chance and act in true collaboration, not competition, with the folks in this room and other knowledgeable wildlife research and wildlife management entities (federal, state, tribal, or local).
2. Work together to leverage resources (hands, funds, minds, knowledge, ideas, data, and information).
3. Ensure that the concepts of wildlife and ecosystem health are incorporated up front in any type of scientific study that you conduct.



Appendix D

Remote Delivery of Biologicals to Wildlife: the Rabies Paradigm

C. E. Rupprecht

Centers for Disease Control & Prevention, Atlanta, GA 30333

D. Slate

U. S. Department of Agriculture, Wildlife Services, Concord, NH 03301

The conundrum of brucellosis persistence among elk and bison in the GYA serves as an example where vaccination may be considered an important wildlife management option. Vaccination of wildlife certainly does not provide a panacea, but it has become an important adjunct consideration in veterinary medicine, public health, wildlife management, and conservation biology. Unfortunately, few success stories exist concerning the utility of vaccinating free-ranging wildlife. Examination of the history and development of global oral rabies vaccination (ORV) programs over the past 50 years, targeted towards meso-carnivores, such as foxes, raccoons, skunks, coyotes, and mongoose, may offer several relevant clues towards the design and delivery of biologicals to other mammals such as bison and elk.

The system of trapping, vaccinating with inactivated biologicals, and releasing animals was used for multiple wild species on several continents, but offered logistical and economical challenges. The original rationale towards ORV grew by inference after several interrelated observations: 1) the epidemiological realization of important wildlife reservoirs; 2) the limitations of population reduction and other available management options as a means of economical, widespread, and long-term control; 3) the creation of safe and effective rabies biologicals; and 4) the successful mass application of parenteral vaccines in the control of canine rabies as a powerful public health tool.

The first generation ORV encompassed modified-live rabies viruses that were effective by the oral route when delivered in bait. Several parameters were necessary in the development of compatible baits, delivery systems, and vaccines, which shaped their concomitant evolution from the 1960s throughout the latter part of the 20th century (Tables D-1, D-2). Progress to date has involved the development of second generation highly attenuated rabies virus and recombinant, pox-vectored vaccines. Vaccine-laden baits are distributed in suitable habitats by hand while walking or driving or by air via helicopters or low flying fixed-wing airplanes. Im-



pressive results have been realized by the widespread elimination of rabies among carnivores in Western Europe, and the virtual disappearance of fox rabies in southern Ontario.

In the United States, the ORV program has grown from the distribution of less than 5,000 baits by hand on one off-shore island during 1990, to more than 11 million doses administered per year in nearly 18 states covering approximately 200,000 km². The current goal (Phase 1) is the geographical containment of raccoon rabies in the eastern United States and gray fox rabies in west central Texas. Phase 2 would entail regional or national elimination of selected rabies virus variants by species, as was accomplished for rabies in coyotes at the Texas-Mexico border from 1995 to 2000. The cooperative National Rabies Management Program is guided by recommendations from multidisciplinary expertise from state and federal agencies and other entities that comprise the Rabies Management Team. The Rabies Management Team functions through ten focus teams covering diverse issues integral to effective ORV. The issues include NEPA compliance, economic analysis, vaccine-bait-biomarker issues, laboratory support and surveillance, air and ground baiting support, ORV evaluation, communications planning, contingency action planning, ORV strategy planning, and research prioritization. The four strategic components of ORV are enhance rabies surveillance, coordinated ORV, use of natural or man-made barriers, and contingency actions. A GIS-based RabID internet reporting system managed at CDC incorporates rabies surveillance samples, which do not involve human or domestic animal exposures and therefore complement traditional passive public health rabies surveillance, and facilitates more informed ORV decisions. Coordinated ORV is tiered to natural land features where practical (e.g., contiguous forest habitats at higher elevation along the Appalachian Ridge) to decrease ORV costs, while increasing the integrity of “immune barriers.” Barrier breaches and other foci are treated through contingency action planning and may also incorporate Trap-Vaccinate-Release and local population suppression to increase effectiveness in containing outbreaks or hotspots.

Several observations from the global ORV program may have particular relevance for brucellosis control in the GYA. These include vaccines having direct penetration of the oral and buccal mucosa without the need for scarification. Moreover, limited passive vaccine transmission from animal to animal has been documented after direct contact, including examples of breeding foxes, raccoon dam-kit interactions, vampire bat conspecific grooming, and commingling between vaccinated and naïve captive deer. Aerosol transmission of rabies viruses in both natural and laboratory settings suggested that such routes could be utilized for immunization by sprays, mists, or fogs applied to animals directly or indirectly in the environment. Such experimental and epidemiological precedence could be utilized in comparative vaccination strategies, exploiting seasonal, social, and other biological and ecological attributes as related to GYA ungulates.

In recent years, other ORV programs have begun to consider the utility of new vaccine design through a reverse genetics system, which offers novel opportunities for both pathogen attenuation and the co-express of foreign genes. Given that many viruses, such as rhabdoviruses, are



transmitted by arthropods, these could be considered as biological agents in gene delivery. In theory, such agents could be adapted to specific arthropods sterilized in the laboratory, as was done in the screw worm eradication program, and be released to specific mammalian hosts, provided basic information is available concerning the genetic markers of protective immunity. Clearly, the focus of ORV and of brucellosis in the GYA differ in a number of important attributes, including etiological agents, target species, and scope. However, results obtained to date in the ORV programs of Europe and North America demonstrate the feasibility of the overall approach; namely, that large numbers of free-ranging wildlife can be reached safely and effectively over broad geographic areas via the remote delivery of veterinary biologicals.

Table D-1. Selected ideal bait and delivery system properties.

1. Maximally attractive for targets, but minimal for non-targets
2. Consumed without caching
3. Compatible with intended biologicals
4. Suitable contact with oral/nasal/gastrointestinal/mucosal surfaces
5. Production ease, storable, and low cost
6. Incorporation of bio-markers (e.g., tetracycline)
7. Maintain integrity and attractiveness

Table D-2. Biological considerations for compatibility of bait, vaccine, and delivery.

1. Biologicals should be formulated to immunize specifically by the intended routes (oral, parenteral, etc.)
2. Stable, apathogenic properties selected for target and non-target species should have low possibility of reversion
3. Active excretion should be minimal
4. Prolonged potency during storage and environmental conditions
5. Production ease and low cost
6. Markers to define product from wild-type agents



Appendix E

History and Current Status of Diagnostic Tools

Klaus Nielsen

Animal Diseases Research Institute, Canadian Food Inspection Agency, Ottawa, Ontario, Canada

History

Late in the 19th century, a number of British soldiers in Malta became ill with an unknown disease. The symptoms were severe influenza-like and very debilitating. Sufficient mortalities occurred to cause the British government to establish the Malta Commission, led by a Major Bruce. Eventually, the disease was linked to consumption of raw dairy products from local goats and the causative organism was established to be a coccobaccillus that eventually ended up wearing Dr. Bruce's name. The Commission findings were published in 1888 constituting the first published report of Malta fever, now known as brucellosis caused by *B. melitensis*. As of the end of July 2005, there are more than 10,000 reports on brucellosis in the literature.

The next major advances were the isolation by Bang, a Danish veterinarian, of *B. abortus* from aborting cattle in 1898 and the first description of a serological test for Malta fever by Wright and Smith. This test was a tube agglutination test that eventually was called the Wright test. In 1909, the complement fixation test was described by M'Fadyen and Stockman and then things were quiet until 1930 when Buck developed *B. abortus* strain 19 vaccine (S19). Over the next few years, a number of advances relevant to the diagnosis of brucellosis were made (Table E-1).

The sum of these findings led to the current knowledge of diagnostic tests. There are three basic ways to diagnose brucellosis. The most cost-effective methods are by the use of serological tests; however, demonstration of the presence of the causative organism and clinical diagnosis are very useful tools.



Current Diagnostic Tests for Brucellosis

Bacteriological isolation and identification

- Isolation and biochemical identification of the organism is the “gold standard.”
- This also allows for identification of vaccine strains.
- This is generally a prolonged procedure that requires biocontainment level 3 facilities and highly trained personnel.
- Requires the shipment of infected materials, tissues or fluids, under very stringent conditions.
- It is generally expensive to maintain media, phages, antisera and quality control.
- It poses a health hazard to personnel in the field and in the laboratory.
- Few countries have this capability.

Identification using nucleic acid technology

- Various polymerase chain reaction (PCR) schemes have been developed for the identification of *Brucella* spp., including AMOS, BaSS, and HOOOF-Print - all developed by the U. S. Department of Agriculture.
- PCR is relatively rapid, less expensive, and less dangerous.
- It does not require biocontainment; however, usually a special laboratory is used to avoid cross contamination.
- PCR requires highly trained personnel and some equipment.
- It cannot distinguish dead from live bacteria and, at the moment, only species, not biotypes, can be identified. Inherent inhibitors may be a problem.
- Allows for identification of vaccine strains.
- Some cross-reactions have been described.

Measurement of cell mediated immunity

- May be done in vivo as a skin test. Requires capture of animals, injection of antigen, and then recapture two days later for assessment of reaction. Not very suitable for most wildlife diagnostics.
- The sensitivity and specificity of the skin test has not been established for wildlife species.
- In vitro measurement of cell mediated immunity usually requires the stimulation of peripheral blood lymphocytes with an antigen other than lipopolysaccharide.



- Lymphocytes are cultured aseptically for 2-6 days with antigen, then tested by either measuring incorporation of a radioactive tracer (tritium) into DNA, or by measuring production of specific cytokines (usually gamma interferon) by a capture enzyme immunoassay.
- The specificity and sensitivity have not been assessed in wildlife species to any extent.



Field trip participants Peter Nara, Keith Aune, Fred DuBray (Intertribal Bison Cooperative), and Rick Willer.

- May be able to eliminate immune response due to some cross-reacting microorganisms.
- Very time consuming and expensive technology. Time sensitive lymphocytes may be a problem.
- For most aspects of infectious disease diagnosis of wildlife, measurement of cellular mediated immunity may be useful but not practical.

Measurement of the humoral immune response

Knowledge about the immune response in wildlife is very limited for obvious reasons. Thus, most of the data available are derived from domestic ruminants, particularly cattle. While this information may not be directly applicable to a wildlife species, it at least provides a starting point for the development and application of suitable diagnostic procedures. The first step is to examine some of the parameters to be considered for a diagnostic test. These parameters include:

- Antigen: preferably containing an immunodominant epitope to which most animals produce an immune response.
- The immune response: measurement of antibody or cell mediated immunity.
- Test accuracy: the ability of the test to distinguish diseased from non-diseased animals each time the test is done.
- Cost: always the lower cost the better. Equipment is a consideration.
- Time: can the test be done chute side in minutes or is it a laboratory test?

- Training: what sort of skill level is required?
- Throughput: high, intermediate or low numbers of tests to be done.
- Politics: whatever is done, someone is going to disagree.

Antigens

- There are six terrestrial species of *Brucella*, divided on the basis of their biochemical characteristics. However, the most important characteristic for a diagnostic test is the presence of smooth or rough lipopolysaccharide, an immunodominant antigen on the surface of the cell.

Immune response

- The cellular immune response does not lend itself well to mass diagnosis in vitro for a number of reasons, including the requirement for time sensitive blood, a laboratory bound assay, the time involved, and the cost. The assays are very good for determination of cross-reactions that are difficult to resolve by other means. In vivo cell mediated immunity may be measured as well but, again, it is time consuming and requires a 24-48 hour period between application of antigen and observing the result. Not really suitable for wildlife usage.
- Therefore, the most suitable diagnostic tool is measurement of antibody. The antibody response depends on a large number of factors. The main ones are route of exposure, dose, age, sex, and pregnancy status. The antibody response of an outbred population of animals will vary considerably in its amplitude, duration, and components. The amplitude is important because the individual animal may not be capable of producing sufficient antibody to exceed a predetermined cutoff value for positivity or the duration of the response may be short or nonexistent as in carrier animals. The antibody isotype response is as important as some isotypes. For example, IgM contains antibody that tends to cross-react and is therefore usually not a good indicator of infection. Other isotypes may be produced at low levels. Therefore, it is important to select the most suitable isotype to measure.

Accuracy

- The sensitivity (the ability of a test to detect diseased animals) and the specificity (the ability of a test to yield a negative result for non-diseased animals) should both be as high as possible. Usually there is a trade-off between sensitivity and specificity; that is, one may be increased at the cost of the other. Therefore, it is important to carefully establish the cutoff value between positivity and negativity. This is usually done by testing a large number of diseased and non-diseased animals and evaluating the results statistically. For some assays, the cutoff may be varied to suit circumstances. For instance, in an area with a high prevalence, the cutoff may be lowered to detect all diseased animals, usually at the cost of some non-diseased animals.



- Repeatability of a test refers to the ability of that test to produce the same result on separate occasions. A test should be able to distinguish diseased and non-diseased animals consistently at a high rate. A test that gives the exact same numerical result each time is also precise. A test can be precise but not accurate.



Field trip participants in Paradise Valley, Montana.

- Validation status of a test is an important newer addition to its definition. A test is no longer acceptable until it has been proven to be correct in the majority of cases. This is done by testing a large number of samples from diseased and non-diseased animals and then comparing the results to a “gold standard” reference test. The latter aspect causes a great deal of problems because almost invariably a new test, with better performance than an older test, will be regarded as incorrect in favor of the old test. The true “gold standard” is proof of disease by isolation of the causative organism and the establishment of Koch’s Postulates.
- Quality control is another newer and very important addition to the test protocol. Unless it can be clearly demonstrated that an assay provides results with reagents of known performance, results for test samples cannot be accepted. Therefore, normally a series of control sera are tested periodically for serological tests. The result obtained with these control sera must fall within limits established by statistical analysis of a large number (at least 30) of previous tests. Most primary binding assays have continuous quality control software that allows for observation of trends as well.
- Standardization of tests has been attempted but has largely failed because most laboratories prefer their own versions of various tests and because reagents used in tests are not universally available. However, even small details such as the protocol used for growing bacteria used as antigen has a major impact in the performance of serological tests. Standardization of tests and their protocols would be a major advance in controlling various infectious diseases.
- Confidence in the results has become a legal as well as a scientific issue. Therefore, most test results are now reported with confidence limits included. For instance, 95% confidence limits mean that 95% of the time the results will be correct while 5% of the time there may be error. This is a generally accepted error margin for biological tests.

Time

- Most diagnostic tests are performed in a laboratory on samples shipped by mail or courier. Therefore, it generally takes at least three days, frequently longer, to get the test results. For wildlife, this poses a major problem as the animals then require re-identification or recapture.
- Some tests can be adapted for use in the field. These tests include various versions of the rapid agglutination tests, or more rapid indirect ELISA and FPA. Field testing is obviously desirable. However, before any test may be used, it will require validation under those specific circumstances. Such tests are also desirable from a cost aspect because shipping has become a major expense.

Cost

- The cost of individual tests varies considerably. Generally, tests that can be adapted to a 96-well format are less expensive as long as there are sufficient samples to fill the plate. This format also has the advantage of being automated which usually results in labor savings.
- The more expensive tests are not necessarily better.
- Other cost considerations are equipment cost and its amortization.

Training

- Performance of all tests require some degree of training, from minimal for rapid agglutination tests to extensive for complement fixation tests and enzyme immunoassays.
- For laboratory tests, perhaps a few trained individuals are needed, but it is possible a much larger number would be required for field testing.

Politics

- An accepted protocol for eradication and control of infectious diseases has been test and slaughter and depopulation. This has been done for situations involving domestic animals; however for wildlife, such measures may not be acceptable for a variety of political reasons.

Serological tests fall into two general categories: the classical or conventional tests and primary binding assays. The conventional tests all require the antibody to be capable of a secondary function: agglutination, fixation of complement, or precipitation. Not all antibody molecules can perform secondary functions and, as a consequence, false negative reactions occur. Alternately, some antibody molecules (IgM in particular) tend to be broadly specific, causing false positive reactions.

The agglutination tests fall into three groups: the regular slow tube agglutination test, the modified slow agglutination tests, and the rapid agglutination tests. The classical slow tube



agglutination test is virtually the same as described in 1898. As the name implies, it takes 24-48 hours to perform; it is prone to false positive reactions due to crossreacting IgM class antibody; and it has been abandoned as a test by most, but not all, countries.

Modified slow agglutination tests generally include a treatment to decrease the contribution of IgM to the agglutination reaction. These treatments include reduction by 2-mercaptoethanol or dithiothreitol and physical removal by rivanol or non-specific interaction elimination by EDTA. Some of these treatments have been adapted to a rapid format tests.

Rapid agglutination tests use a large amount of antigen and neat serum to produce agglutination in minutes. Most of these assays include a treatment to diminish interaction by IgM. The treatments include the use of the antigen at a low pH and a stained antigen to make agglutination easier to observe.

Complement fixation tests are based on the ability of an antigen-antibody complex to activate guinea pig complement thereby depleting complement available to be activated by an indicator system, usually sheep erythrocytes coated with rabbit antibody. This is a very specific and sensitive test, but it has some major disadvantages in that it is technically cumbersome, requires a large number of reagents, and is prone to anticomplementary activity in some sera. The agglutination and complement fixation tests, usually in combination, have successfully been used to eradicate brucellosis in domestic animal in a number of countries.

Precipitation tests have not been widely used for the diagnosis of infection by smooth *Brucella* spp., rather they are most often used to diagnose *B. ovis* infection. The test uses an agar matrix into which wells are prepared. Serum and a soluble antigen are placed in opposite wells and allowed to diffuse. A positive reaction is a visible precipitin band between the wells. A second protocol uses antigen incorporated into the agar matrix such that a ring of precipitin forms around positive wells. This type of test was the first to distinguish *B. abortus* strain 19-vaccinated from infected animals. While there are a number of disadvantages to the conventional tests, they are widely used for diagnosis of brucellosis. The agglutination tests lend themselves well to screening large populations of individual animals while the complement fixation tests are useful confirmatory tests.



Montana Fish, Wildlife and Parks personnel with bison.

The primary binding assays include various formats of enzyme immunoassays, fluorescent particle counting immunoassay, and fluorescence polarization assay. These assays do not require the antibody to perform any function other than binding to its antigen. The assays are readily mechanized and the subjective element of reaction assessment, common to conventional tests, has been replaced by electronic reaction measurement. Additional advantages are that the cutoff point is readily manipulated to suit circumstances and quality control can be managed.

Enzyme immunoassays generally come in two formats, indirect or competitive. Both assays use antigen, usually lipopolysaccharide, immobilized on a polystyrene matrix of 96-well plates. In both assay types, washing is done between each step. In the indirect assay, test serum is added next, followed by a detection reagent, which can be an anti-species immunoglobulin prepared in a different species, a monoclonal anti-immunoglobulin, or a non-specific reagent such as protein A. In all cases, the detection system is labeled with an enzyme and a positive reaction is observed if the substrate/chromogen develops color. In the competitive assay, test serum and a competing antibody, usually a monoclonal antibody specific for an epitope of lipopolysaccharide, are added together followed by an enzyme conjugated detection reagent for the competing antibody, usually an anti-mouse antibody. If addition of substrate/chromogen causes development of color, the reaction is negative. In general, enzyme immunoassays are laboratory tests; however, some formats of the indirect version are available as rapid field tests.

The fluorescent particle counting immunoassay is based on the same principle as the competitive enzyme immunoassay. The antigen is immobilized on beads and the competing reagent is a fluorochrome-labelled polyclonal antibody. This assay has been highly automated for large throughput testing; however, its performance, particularly specificity, was lacking.

Fluorescence polarization assay is a homogeneous assay which is performed without intermediate removal of excess reagents. Basically, fluorochrome-labeled antigen is added to diluted serum and after a very brief incubation period, a result is obtained. The result is based on a shift in the rate of rotation of molecules in solution when their size is altered. Thus a small molecule will rotate rapidly; however, if antibody attaches to it, the rate of rotation is decreased in a measurable fashion. The test is very rapid, may be done in minutes, and is rugged enough for field use. It can be automated for high throughput and it has good sensitivity and specificity.

Summary

Diagnosis of brucellosis is not simple. Ideally, the causative agent should be demonstrated in individual hosts, but that is usually not possible and demonstration of its presence in the herd suffices for action. Individual animal diagnosis is done almost exclusively by serological testing. Therefore, it is imperative that the most suitable tests be used for each circumstance. For instance, the same serological test used for diagnosis in cattle may not be useful for diagnosis



in swine or even small ruminants. Not much information is available on the serological diagnosis of brucellosis in wildlife and, as a result, careful assessment of individual tests in their context is required. Below is a summary of tests used in the diagnosis of brucellosis in cattle. It is a compilation of the range of sensitivity and specificity values published over the years along with a performance index which is the sum of the sensitivity and specificity values.

Table E-1. Tests used in the diagnosis of brucellosis in cattle.

Test	Total number of samples tested	Mean of sensitivities	Mean of specificities	Mean Performance Index
PCFIA (n = 6)	2,436	91.8	46.7	138.5
Card (n = 11)	6,434	91.0	55.2	146.2
Rivanol (n = 12)	4,845	89.6	63.1	152.7
RBT (n = 11)	12,146	81.2	86.3	167.6
TAT (n = 14)	9,534	75.9	95.7	171.6
CFT (n = 38)	28,537	89.0	83.5	172.5
2-ME (n = 4)	7,693	88.4	91.5	179.9
CELISA (n = 14)	15,865	97.7	90.5	188.2
IELISA (n = 37)	60,985	96.0	93.8	189.8
BPAT (n = 15)	60,634	95.4	97.7	193.1
FPA (n = 7)	39,934	97.5	98.9	196.4

Table E-2. History of brucellosis from 1888-2005.

Year	Event
1888	<i>B. melitensis</i> was described as the cause of Malta fever by Bruce.
1898	<i>B. abortus</i> was isolated from an aborting cow by Bang.
1898	First serological test was described for Malta fever by Wright and Smith.
1909	The complement fixation test was described by M'Fadyen and Stockman.
1930	<i>B. abortus</i> S19 vaccine was developed by Buck.
1952	<i>B. ovis</i> was isolated by MacFarlane et al.
1957	<i>B. neotoma</i> was isolated by Steomer and Lacknam.
1963	The complement fixation test was standardized by Hill.
1967	The rose bengal/card test was developed by Morgan.
1968	<i>B. canis</i> was isolated by Carmichael and Kenny.
1968	Differentiation of vaccine from field strain antibody was done by Diaz et al.
1970	Indirect ELISA developed by Carlsson et al.
1984	Buffered antigen plate agglutination test described by Angus and Barton.
1984	The structure of smooth lipopolysaccharide elucidated by Caroff et al.
1985	Particle counting fluorescence immunoassay described by Jolley.
1989	Competitive enzyme immunoassay developed by Nielsen et al.
1990	First PCR for <i>Brucella</i> described by Fakete et al.
1991	<i>B. abortus</i> RB51 vaccine was developed by Schurig et al.
1996	Fluorescence polarization assay was developed by Nielsen et al.
1996	<i>B. maris</i> was isolated by Foster et al.
2000	AMOS PRC was developed by Ewalt and Bricker.
2003	BaSS PRC was developed by Ewalt and Bricker.
2003	HOOF-print PCR developed by Bricker et al.





Appendix F

Perspectives for Eradication of Brucellosis in Yellowstone Bison

By Alexander Denisov

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The importance and urgency of the problem of bison brucellosis in YNP is beyond question. In our opinion, any measures directed to eradication of brucellosis should be based on animal vaccination. 40-year experience of Russian scientists has proved that specific prophylaxis is one of the most perspective trends in settling the problem. Therefore, the experience gained in the years of brucellosis control formed a base for initiation of cooperative Russian-American studies directed to decreasing the infection with brucellosis in bison of the Yellowstone National Park and reducing the risk of its transmission to wild and farm animals.

These studies started 2 years ago and are going on in the framework of the International Science and Technology Center project. The project title is “Comparative studies of immunobiological properties of live brucellosis vaccines.” The project objective is selection of the safest and most effective vaccine for specific prophylaxis of Yellowstone bison brucellosis.



To achieve the goal, the following tasks were fulfilled:

1. renovation of the Vivarium at Kazan Veterinary Institute in compliance with Russian and US standards (not scientific task, however, of great importance for the project implementation);
2. comparative study of immunological properties of several live brucellosis vaccines and selection of the safest and effective one;
3. selection of the most effective adjuvants for enhancing immunogenic properties of live brucellosis vaccines; and
4. development and testing of ballistic method for the vaccine delivery in cattle.

Today the renovation of the vivarium in Kazan Veterinary Institute is completed. All the facilities meet the International requirements to maintenance of experimental animals and the building is prepared for conducting experiments with small laboratory animals including SPF animals as well as with BSL3 pathogens.

Both Russian and American vaccines present in 3 different forms (S-, R- and SR-form) are used for comparative studies. They include *B. abortus* RB51, *B. abortus* 19 (from USA) and *B. abortus* 82, *B. abortus* 82-PS (penicillin sensitive) and *B. abortus* 75/79 (from Russia). Today studies of cultural, morphology and biochemical properties, antigenic properties, persistence duration, residual virulence and contagiousness and optimal immunizing dose for guinea pigs of all tested vaccines have been completed. The rest studies of abortogenicity, immunity duration and efficiency are going on and will be completed in the near future.

The next direction of our research is assessment of the potential to use adjuvants for enhancing immunogenic properties of live brucellosis vaccines. For these studies 5 adjuvants were selected: tumor necrosis factor, polyoxidonium, potassium thiosulfate (from Russia), larifan (from Latvia) and RAS (from USA).

Our studies demonstrate that some of the adjuvants increase the synthesis of R-antibodies and production of S-antibodies as well. High levels of R- and S- antibodies maintained within 6 months. But we failed to reveal the reliable difference in the levels of cellular immunity between control and experimental animals after 3 and 6 months.

The last direction of our research is connected with development of ballistic method for the remote delivery of brucellosis vaccines. Among a great number of bullets of different kinds, we found the bullet of 5.6 mm caliber developed by Russian scientist Komarov V.A. We have modified this bullet to make it suitable for vaccine loading. The data obtained proved the stability of ballistic characteristic of the modified bullets firing from a distance of 100 m. To avoid the toxic effect of lead (the bullet material) on vaccine cells the inner surface of the bullet was covered with special protective substance. The efficiency of the ballistic method was demonstrated in the field experiments on the live animals (heifers) in comparison with i/m



vaccine administration using *B. abortus* 82 and 19 vaccines. We have shown that Komarov's bullet does not cause an excessive tissue trauma at injection sites, has limited penetration in animal tissues (5-6 cm), readily and completely releases the vaccine into animal tissues, and doesn't hurt the animals. The immune response on both vaccines was revealed by serological reactions (RA, RBT and CFR) and antibodies detection. Titers of antibodies in animals vaccinated intramuscularly were higher than at ballistic method of vaccination. The most important outcome of these studies is that we have demonstrated the potential of vaccination of animals with live brucellosis vaccine using ballistic method from the distance 100 m. This method works and stimulates immunological response in animals.





Appendix G

Russian-American Collaboration on Brucellosis Study

By Roman V. Borovick

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Up to middle sixties in Russia vaccine obtained from *B. abortus* strain 19 of American origin had been widely used for animals immunization. The grave disadvantage of the vaccine is that it triggers agglutinin elaboration while immune maturation. Thus it proves impossible to differ sick animals from immunized animals and by effective discarding of infected animals bring the herd as a whole into a healthy state. The vaccine from strain 82 developed by Prof. K.M.Salmakov¹ not only possesses high immunizing power, but also does not induce agglutinin synthesis. So, sick animals can be removed. The use of this vaccine allowed for almost complete elimination of *B. abortus* infections in cattle in Russia in ten years.² Thus the proposed by Russian epidemiologist L.V. Gromashevsky triad-concept for termination of epizootic is implemented. The concept³, which was given the name of the 4th rule of epizootology, is as follows:

1. Eradication of the infection source;
2. Distortions in transmission of infection; and
3. Effective vaccination.

¹“Live vaccine against *Brucella abortus* from 82 strain”. K.M. Salmakov. Veterinary(in Russian), 1975, 7, pages 43-45

²“Combating brucellosis in cattle with the use of strain 82 vaccine” V. M. Avilov, K. M. Salmakov, A.A. Novitsky. Veterinary(in Russian), 2000, 3, pages 3-7;

³T. Rożniatowski, I Z. Żoltowski *Wojna Biologiczna. Groża rzeczywistości*. Warszawa, 1957, p.73



The problem of elimination of *B. abortus* infections in cattle in GYA is caused by the fact that each of the listed above goals can hardly be achieved in work with large wild artiodactyl animals (bison, elk, deer, etc). So the use of highly efficacious vaccine should be staked on.

In addition to that, the process of infection transmission is still far from being studied. In particular, the part, small rodents play, has not been estimated to proper extent⁴; or the estimation is made with use of techniques which are not enough sensitive or involving test models. The part, which Ground squirrel, in large numbers dwelling in the bison inhabited areas, plays in transmitting of the infection has not been studied properly either. There is no data in American publications about *B. abortus* pathogenic strains found in these rodents. PCR analysis should be performed in case of *B. abortus*-infections, where the concentration of *B. abortus* in blood is extremely low and runs out quickly and thus allows for favorable, but wrong conclusions.

The very source of infection should be eliminated – that is removal of sick animals and forming healthy insusceptible to infection cattle herds. It can be achieved by use of the effective vaccine obtained from strain 82 in Russia and by use of novel, nontraditional methods of delivery – ballistic delivery or self-vaccination (via food, with the use of bloodsucking ticks and other methods).

There is hope, that joint efforts of American and Russian scientists will help to solve the task, the implementation of which has been successfully started in the framework of ISTC international Project - # 2434.

⁴Januszewski, M.C., Olsen S.C. , McLean, R.G., Clark, L., Rhyan, J.C. 2001. Experimental injection of nontarget species of rodents and birds with *Brucella abortus* strain RB82 Vaccine. *Journal of Wildlife Diseases*, 37(3):532-537





Appendix H

Cattle Brucellosis in Russia and its Specific Prophylaxis

By Konstantin M. Salmakov (summarized from Dr. Salmakov's plenary presentation by Glenn Plumb)

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Immuno-prophylaxis is one of the most important tools for control and in many cases, elimination of infectious disease. Live vaccines obtained from agglutinogenic *B. abortus* 19 and *B. melitensis* Rev 1 (USA) strains have been most widely recognized in the world. As for dissociated strains, live vaccine based on *B. abortus* 82 (Russia, ARVI, Kazan) is being currently applied in Russia as an officially approved preparation. The 50-year experience of fighting cattle brucellosis in Russia demonstrated the exceptional role of specific prophylaxis in the recovery of livestock. In the USSR, during 1954-1970, the use of strain 19 vaccine in cattle of both sexes and all ages allowed sharp reduction in the number of clinical cases of brucellosis, as well as narrowing the infection foci and many times decreasing epizootological and epidemiological indices of the disease. At that time, sharp debate occurred on the expedience of this vaccine application. The main argument against is lengthy post-vaccinal sero-positivity that masks a real epizootological situation in the livestock regarding brucellosis and impedes its recovery.

This was the reason to stop re-vaccination of cows with strain 19 vaccine in 1970. Indeed, 2-repeated immunization with this vaccine of only heifers at the age of 3-6 months and just before insemination did not assure future protection of adult animals against brucellosis and resulted in considerable increase of the number of infected cattle at different farms. Therefore, the urgent need arose for a alternative brucellosis vaccines that did not cause prolonged sero-positivity in immunized cattle. A search was conducted by different Russian research veterinarians, resulting in a number of new *B. abortus* vaccine strains. The most promising among them was strain *B. abortus* 82 developed in 1960 by Professor Salmakov (ARVI, Kazan). Results of the



vaccine author's studies were confirmed by the studies conducted by other research institutes. Strain *B. abortus* 82 was repeatedly examined in jointly conducted experiments. Government testing of this vaccine conducted on laboratory animals and cattle in experimental and industrial conditions have established the presence of weak agglutinogenic and pronounced immunogenic properties. In 1974, strain 82 vaccine was used in 34 regions of Russia on approximately 30 million animals in extreme epizootic conditions; immunized were animals of all ages. After that, 6-9 million animals were annually inoculated with this vaccine.

Broad application of the strain 82 vaccine, providing a strong immune background and possibility of early post-vaccinal diagnostics (after 3-6 months compared to 2-3 years after strain 19), made it possible to reduce epizootic tension concerning cattle brucellosis in Russia. High epizootic efficiency achieved has encouraged the Head Veterinarian Directorate at the Ministry of Agriculture to approve the live strain 82 vaccine for use in veterinarian practice for fighting cattle brucellosis. For over 30 years, Biological industrial complex in Shchelkovo (Moscow region) has been producing dry strain 82 vaccine successfully applied in many regions of Russia as integral part of the veterinary-sanitary program for control of cattle brucellosis. By the end of 2004, after taking special measures including application of the vaccine in cattle, the number of places with brucellosis was decreased 74 times compared to 1974 (Figure H-1).

Positive results were also achieved at application of the vaccines in other animal species, (e.g., reindeers, marals, yaks, buffalo, zebu, etc.). The observations made during the experiments and in industrial production have demonstrated that in conditions of direct threat of infection, vaccination of animals is not a complementary but is the critical measure that assures a reliable anti-brucellosis protection and recovery. With the use of strain 82 vaccine, the problem of brucellosis in many regions of Russia has been solved. At that, a considerable achievement is the absence of animal- transmitted brucellosis infections in humans.

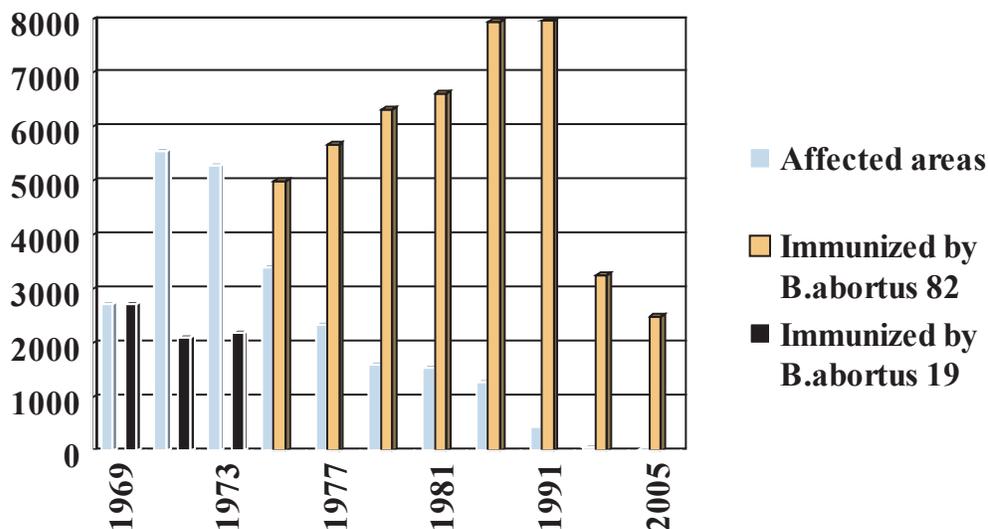


Figure H-1. Epizootic situation of cattle brucellosis in Russian Federation after application of vaccines *B. abortus* 19 and 82.





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