Bovine Brucellosis

This course is designed to provide updated information on the major domestic diseases for which Veterinary Services (VS) has program responsibility. It will provide information on surveillance, disease control and eradication for these diseases. It will also give an overview of the duties of a field Veterinary Medical Officer (VMO) as a support worker of VS animal disease programs and how they interact with other units in APHIS.

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1. DISEASE INFORMATION

a. Agent
Brucellosis is a contagious disease caused by various species of Brucella, a Gram negative rod in the family Brucellaceae. Brucellae are facultative intracellular pathogens, mainly proliferating inside cells of the macrophage lineage. Each species of Brucella is typically associated with a limited number of hosts but occasionally infects other species, particularly those in close contact. Brucella abortus, the most common cause of brucellosis in domestic cattle, infects cattle and bison. It is also found occasionally in sheep, goats, domestic buffaloes, African buffaloes, water buffaloes, yaks, camels, horses, dogs, some African antelopes, and various Cervidae including elk and deer. Infected ruminants can shed bacteria long-term or lifelong.

Other Brucella species that occasionally infect cattle include B. melitensis, ordinarily found in sheep and goats, and B. suis, found mainly in pigs.

The optimal conditions for the survival of Brucella are high humidity, low temperatures, and no sunlight. Live bacteria have been found in soil for up to 125 days, in carcasses and organs for up to 135 days, and in blood stored at 4 °C (39 °F) for 180 days.

b. Transmission
Brucellosis is usually transmitted when an animal ingests bacteria shed in birth or abortion products. B. abortus can infect an animal through the mucus membranes, conjunctivae, wounds, or skin. Large numbers of bacteria are found in the placenta, fetal fluids, and vaginal discharges after abortions and sometimes after full term pregnancies. Organisms are also found in the udder and its associated lymph nodes and are shed in the milk. B. abortus can be found in the fluids of Brucella-associated hygromas and is occasionally detected in the urine, feces, or semen. Venereal transmission can occur but is rare. B. abortus can be transmitted on fomites.

Although cows typically abort only their first pregnancy after infection, most animals become chronic carriers. In some cows, the bacteria recolonize the uterus and mammary gland in later pregnancies and bacteria are shed intermittently in the milk, though in smaller numbers than during the initial infection.

The mammary gland can also be infected by direct contact with B. abortus, B. melitensis or B. suis on the hands of farm workers, with these species then shed in the milk.

B. abortus is zoonotic. Humans become infected by ingestion, inhalation, or direct contact through the mucous membranes or breaks in the skin. Veterinarians are usually infected when they assist during births or are accidentally injected with the Strain 19 vaccine. Carcasses are the usual source of exposure for meatpackers, and the general population typically ingests B. abortus in unpasteurized milk or milk products. Aerosol transmission has been documented in laboratories and slaughterhouses.

c. Clinical signs
Brucellosis is usually asymptomatic in nonpregnant female cattle. In pregnant cows, B. abortus causes late term abortions, typically between the fifth and ninth months, and stillbirths. Some calves are born at full term but are weak or die soon afterward. The placenta is often
retained and secondary metritis can occur, sometimes leading to permanent infertility. Milk yield is often reduced but, in uncomplicated abortions, the general health of the cow is not affected. Subsequent pregnancies are usually carried to term. Infected bulls sometimes develop acute or chronic orchitis and testicular abscesses. After long-term infections, cattle may develop arthritis. In some tropical countries, brucellosis tends to cause hygromas on the joints of the legs, particularly the carpal joint.

*B. abortus* infections occasionally occur in other species kept in close contact with cattle. In other ruminants, brucellosis resembles the disease in cattle. In horses, *B. abortus* can cause inflammation of the supraspinous or supra-atlantal bursa; these syndromes are known, respectively, as fistulous withers and poll evil. The bursal sac becomes distended with a clear, viscous, straw-colored exudate and develops a thickened wall. The sac can rupture, leading to secondary inflammation. In chronic cases, nearby ligaments and the dorsal vertebral spines may become necrotic. *Brucella*-associated abortions are rare in horses, but have been seen.

**d. Epidemiology**
The important reservoirs of *B. abortus* in the United States include cattle, bison, and elk. Infections in unusual host species, such as sheep or goats, are not thought to be important in maintaining bovine brucellosis. *B. abortus* is usually introduced into a herd via an infected animal or, more rarely, in semen from an infected bull. The greatest risk of transmission occurs when an infected cow aborts or gives birth.

Ruminants usually abort only their first gestation after infection. In newly affected herds, 30-80% of the pregnant cattle may abort. Deaths are not usually seen in adult animals. Although some cattle are resistant to infection and a few infected cows will recover, however most become chronic carriers.

Natural resistance to *Brucella abortus* exists among cattle and can be increased by selective breeding. In one study, 20% of unvaccinated cattle were resistant to brucellosis challenge before selection but 58.6% of their progeny were resistant after one generation of selective breeding. Resistance appears to be controlled by two or more interacting genes.

The eradication of *Brucella abortus* from the U.S. is complicated by its presence in wild bison and elk in the Greater Yellowstone Area (Yellowstone National Park and its surrounding regions). Although *B. abortus* does not seem to exist in wild animals in other parts of the country, more than half of the bison in Yellowstone National Park are seropositive. Moose in the Yellowstone area do not seem to be infected. In 1990, researchers demonstrated that infected bison could transmit *Brucella abortus* to cattle by direct contact under experimental conditions. Transmission has been difficult to prove under natural conditions, but epidemiologic investigations suggest that bison and elk may have spread brucellosis to domestic cattle herds in Wyoming, North Dakota, and Idaho, as well as to horses with fistulous withers in Wyoming.

Transmission between wild bison and domestic cattle usually occurs when these animals are allowed to mingle. Until 1988, few bison left Yellowstone National Park, and those animals that did were hazed back or killed. However, during the winter of 1996-1997, severe weather and large numbers of bison resulted in a food shortage. At least 1300 bison starved to death inside the park and more than a thousand animals left the park to search for food. There is a risk that
this will occur each winter. In 2002, approximately 934 bison migrated out of the park but were hazed back, and another 262 animals were captured and tested for brucellosis. Of these 262 bison, only 63 animals (24%) tested negative and were released; the remaining 199 bison (76%) tested positive and were slaughtered.

Although elk in the Yellowstone area are also infected, elk are less likely to transmit brucellosis than bison. Elk are less gregarious, particularly around the time when they calve. Wild elk usually calve in seclusion, eat the placenta and birth fluids to avoid attracting predators, and remain separated from the herd for several days. However, the risk of transmission is increased at artificial elk feeding grounds, where animals are more concentrated. Approximately 25,000 elk winter on the National Elk Refuge in Jackson, Wyoming and on 22 other Wyoming feedgrounds. Vaccination of elk with Strain 19 at one feeding ground was able to decrease the percentage of infected elk from 46% in 1985 to 23-26% in 1993-1996.

Predators and scavengers are, on rare occasions, transiently infected with *Brucella abortus* but do not generally shed bacteria. These animals can disseminate brucellosis by dragging infected carcasses or tissues from abortion sites.

### e. Diagnosis

Brucellosis should be considered in all abortions, particularly when there are multiple late-term abortions in a herd. Although this disease resembles other causes of abortion in individual animals, the herd history may be suggestive. In naïve herds, it may be recognized as an abortion storm, with abortions in 30-80% of pregnant animals. Where brucellosis has become endemic, the herd will probably have only sporadic signs and cows may abort their first pregnancies. The uterine lesions after an abortion are variable. Cows may have mild to severe endometritis, the regional lymph nodes may be inflamed, and the mammary gland may contain lesions. Some aborted fetuses look normal, but others have subcutaneous edema, blood-tinged fluid in the body cavities, an enlarged and discolored orange-brown liver, fibrinous pleuritis, or focal pneumonia. The placenta is typically thickened, edematous, and yellowish-grey and is sometimes covered by an exudate.

Although brucellosis is most likely to be recognized in pregnant cows, clinical signs, such as unilateral or bilateral swelling of the scrotal sacs in bulls or inflammation of the testes and epididymis are seen occasionally. Hygromas may be found at slaughter in both sexes on the knees, stifles, hock, angle of the haunch, and between the nuchal ligament and the primary thoracic spines.

For a presumptive diagnosis, smears can be made from the placental cotyledons, vaginal discharge, and fetal liver, lung, and abomasal contents. The slides are stained with a modified Ziehl-Neelsen, Köster’s, Gram, or Macchiavello stain or with immunohistochemistry. Aggregates of weakly acid-fast rods or immunostained bacteria suggest brucellosis. Other bacteria, including *Coxiella burnetii* and *Chlamydia psittaci*, can look similar to *Brucella* and some species such as *Yersinia enterocolitica* can be cross-reactive in immunostained preparations.

The definitive diagnosis depends on culture or serology. *Brucella abortus* can be cultured from the cotyledons of the placenta, vaginal discharge, fetal tissues or abomasal contents, hygroma fluid, milk, and colostrum. In most cows, bacteria are no longer shed from the genital tract once the uterus has completely involuted; however, they can often be found in the secretions of
non-lactating udders. At necropsy, *B. abortus* can be cultured from the mammary gland, uterus, and supramammary and internal iliac lymph nodes of cows. In males, the testes, epididymis, seminal vesicles, accessory glands, and external and internal iliac lymph nodes are most likely to be positive. In either sex, the parotid, mandibular, and retropharyngeal lymph nodes can be cultured. Vaccine strains of *Brucella abortus* can be differentiated from field strains (by serology, culture or both). Polymerase chain reaction (PCR) and DNA probe tests have also been developed.

A variety of serological tests are used for screening herds and testing individual cattle. The buffered *Brucella* antigen tests, which include the rose Bengal test and the buffered plate agglutination test, are the tests prescribed by the World Organization for Animal Health (OIE) for international trade. Cross reactions can occur in serologic tests with Gram negative bacteria including Francisella, Campylobacter, Salmonella, Pasteurella, Yersinia enterocolitica, and *Escherichia coli* 0157:H7. Serological tests can also be used to diagnose *B. abortus* infections in other species such as bison, buffalo, and elk, but the specific test should be validated for each species. In dairy cattle, bulk milk samples can be tested with an indirect ELISA or *Brucella* milk ring test (BRT). The brucellin skin test is sometimes used to diagnose brucellosis or distinguish infected cattle from those with cross-reacting antibodies.

**f. Prevention and control**

Infected herds are identified by a variety of surveillance methods, including the brucellosis milk surveillance tests (BMSTs), which test bulk milk samples from dairies; the market cattle and bison identification (MCI) program, which tests animals sent to slaughter and to markets; pre-movement tests on farms and ranches, area testing, and diagnostic tests on herds with clinical signs. Additional herds are also found during epidemiologic investigations of infected herds. As almost all human cases of brucellosis are zoonotic, the eradication of brucellosis in animals can greatly reduce the threat to humans. When handling infected animals or carcasses, the risk of infection can also be decreased to some extent with good hygiene and protective clothing including gloves, rubber boots, a face mask, and impermeable clothing. Pasteurization can eliminate *Brucella* species from milk and milk products. Potentially infected material should be treated with caution and disposed of properly to prevent human infections. Particular care should be taken in the laboratory, during culture and other procedures that amplify the number of organisms. The OIE and Centers for Disease Control and Prevention (CDC) recommend biosecurity containment level 3 conditions be used during laboratory culture.

*Brucella* species are susceptible to numerous disinfectants, including commercial disinfectants, 1% sodium hypochlorite, 70% ethanol, iodine/alcohol solutions, glutaraldehyde, and formaldehyde. *Brucella* can also be inactivated by moist heat of 121 °C (250 °F) for a minimum of 15 minutes, or dry heat of 160-170 °C (320-338 °F) for at least 1 hour.

There is no practical treatment for cows infected with *Brucella abortus*; however, two vaccines – Strain 19 and RB51 – provide good immunity to moderate challenge by field strains. RB51 has replaced Strain 19 for most purposes.

**g. Public health consequences**

*B. abortus* is highly pathogenic for humans. Brucellosis is almost always a zoonotic disease and person-to-person transmission is extremely rare. Infections with *B. abortus* are usually an
occupational disease in farm workers, veterinarians, and meatpackers, and are acquired from unpasteurized milk and other dairy products in the general population. The Strain 19 vaccine can also cause disease, if it is accidentally injected. The virulence of the RB51 vaccine has not been determined; no human infections have been reported, but this vaccine should also be handled with caution.

In humans, brucellosis can be either an acute febrile disease or a chronic infection with variable clinical signs. The incubation period for human brucellosis is usually five days to two months, but is occasionally longer. Subclinical and unrecognized infections occur frequently. Some infections resemble influenza, with fever, anorexia, myalgia, severe limb and back pains, and marked sweating and fatigue. In humans, unlike domestic animals, abortion is not a feature.

Many untreated cases resolve spontaneously after 2 to 4 weeks. Other patients develop undulant fever, with the fever and clinical signs recurring and receding at approximately 10 day intervals. Most people with this undulant form recover completely in 3 to 12 months, but some can develop complications including arthritis, spondylitis, anemia, leukopenia, thrombocytopenia, uveitis, optic neuritis, endocarditis, granulomatous hepatitis, and neurologic signs. A chronic form, in which the symptoms continue for more than a year, is also seen. The symptoms of the chronic form are poorly understood, but may include chronic fatigue, depressive episodes, and arthritis. Hypersensitivity reactions can also mimic the symptoms of brucellosis. By some estimates, fewer than 10% of human brucellosis cases are recognized and reported because the clinical course is so variable.

In humans, brucellosis is treated with antibiotics. Relapses can occur months after the initial symptoms, even in successfully treated cases. The mortality rate is low, even when the disease is not treated; in untreated persons, the case fatality rate is less than 2%. The vaccine strain RB51 is very sensitive to tetracycline and prophylactic antibiotics should be considered after accidental inoculation.

2. HISTORY OF BOVINE BRUCELLOSIS AND ITS CONTROL PROGRAMS

Brucellosis or “contagious abortion” has been recognized as a serious animal problem since the 1840s. In 1897, the Danish veterinarian Bernhard Bang isolated the causative organism, Bacillus abortus (which later became Brucella abortus), from cattle; for many years, this disease was known as “Bang’s disease.” A British army surgeon, Sir David Bruce, first found the organism in human patients on the island of Malta, prompting two of the names for this disease in humans - brucellosis and Malta fever. The third term, undulant fever, comes from the waxing and waning nature of many human infections.

Brucellosis was the most significant livestock disease of the early 20th century. Although Brucella caused only sporadic abortions in some herds, its first appearance in a newly infected herd was often heralded by abortion storms, a drop in milk production and, occasionally, sterility in some animals. Humans became infected when they drank unpasteurized milk or were exposed to infected animals. In 1938, more than 4,000 human cases of brucellosis were reported in the U.S., many of them in slaughterhouse workers. By 1947, that number had risen to over 6,000 cases (4.4 cases/100,000 population).
During the 1930s, with the Great Depression and severe droughts causing hardship for farmers, the government established an emergency program to reduce cattle numbers and stimulate economic recovery. To some states, this seemed to be a golden opportunity to reduce the level of brucellosis. In 1934, a cooperative state-federal brucellosis eradication program was established as part of the emergency program. At the time, the incidence of brucellosis was 11.5%. Initially, the program concentrated on brucellosis in cattle. It offered herd testing and slaughter of reactors, with indemnity payments to their owners. Many states began to require that breeding and dairy cattle originate in brucellosis-free accredited herds or test negative before they entered the state, and some states established area testing programs.

At the same time, attempts were being made to develop a vaccine for cattle. Some of these early vaccines contained live organisms that persistently infected cows, a misfortune that helped prompt the federal licensing of vaccines. However, in 1936 the 19th strain of Brucella abortus isolated from the milk of a Jersey cow, protected calves from infection during a field trial, did not cause persistent infections, and did not spread to other calves. Trials with Strain 19 were so successful that this vaccine became established as the vaccine for cattle worldwide. By 1941, as a result of the test and slaughter program, as well as vaccination, the incidence of brucellosis had been reduced to 2.4%.

Although WWII slowed the progress of eradication, the program was rejuvenated after the war. In 1947, the first Uniform Methods and Rules (UM&R) were established and a market cattle testing (MCT) program was set up to find infected herds. The eradication efforts were aided by the development of new tests including the milk ring test and complement fixation. As a result of the brucellosis eradication program and the widespread pasteurization of milk, the number of human cases of brucellosis began to drop.

For many years, experts had debated whether to control brucellosis by vaccination alone, or to eradicate the disease. In 1954, the brucellosis program became an accelerated eradication program. With at least 124,000 infected cattle herds still existing nationwide, a target date of 1975 was set for complete eradication. At this time, swine brucellosis eradication still lagged far behind bovine brucellosis. However, the changing pattern of human infections soon focused attention on pigs. Before 1960, most human cases of brucellosis had been caused by Brucella abortus. As the number of infections in cattle declined, brucellosis increasingly became an occupational disease related to pigs; from the mid-1960s to the early 1970s, most human infections were caused by B. suis. In the 1960’s, alarmed at the high incidence of brucellosis among abattoir workers, California officials threatened to accept slaughter hogs only from states that were brucellosis-free. This threat worried the pork producers of the Midwest and, in 1961, the swine brucellosis eradication program was established. Still hoping to eradicate brucellosis by 1975, the brucellosis committee also became concerned about infections in domestic bison, as well as the wild bison in Yellowstone National Park.

However, the target date of 1975 passed and in 1976, brucellosis was not only still present in the U.S., but was spreading faster than the program could clean up infected herds. One problem was that some federal funds had been diverted to other animal health programs. In 1978, a brucellosis technical commission reported that "control leading to eradication is biologically feasible" and recommended changes in the program, including increased testing. At the time, 7,483 infected herds still existed - undoubtedly an underestimate, as some areas still had no real surveillance to find infected herds. In 1986, a five-year swine brucellosis
eradication plan was approved and, in 1989, APHIS began another program, the Rapid Completion Plan (RCP), to accelerate the eradication efforts. 20 1990 marked a major milestone in the bovine brucellosis program; the number of infected cattle herds fell below 1,000 and surveillance programs existed in all states. 19

In another 10 years, the eradication program has come close to success. 14 Currently, the domestic cattle and pig herds of most states are free of brucellosis. Only Texas, Arkansas, Florida, and Louisiana still contain brucellosis-infected domestic pig herds and only Missouri and Texas have infected cattle herds. 21,22 Cases of human brucellosis are now rare in the U.S; fewer than 0.5 cases per 100,000 are seen each year. 18 Occupationally related infections have decreased since the early 1970s. 8 In 1997, APHIS began the Brucellosis Emergency Action Plan (EAP), as a supplement to the RCP. 20 As a result of the EAP, all brucellosis surveillance and management of new cases are now conducted as emergencies.

Brucellosis is now found mainly in wild animals in the U.S. As the last infections in domestic animals are eliminated, APHIS will shift its attention to surveillance efforts and attempt to eradicate brucellosis in bison and elk in Yellowstone National Park, other wildlife, and exotic livestock. 15,23 The current program prevents disease transmission by bison management, but APHIS eventually plans to eliminate the disease from wild bison. 12,15 Methods to control brucellosis in wild and feral swine are also being studied. 23 Research is continuing on the RB51 vaccine, which APHIS expects to use in Yellowstone bison. 23

3. CURRENT CONTROL PROGRAM

The brucellosis program is based on three components - surveillance, prevention, and eradication. 24 The bovine brucellosis program classifies states into Class B, Class A, and Class Free states based on the herd infection rate. Class C status no longer exists. 13 Although a class usually applies to the entire state, a state can request a two-area classification. 17

Each state must submit an annual report on its brucellosis control activities, including surveillance information and the herd infection rate. 17 Herds infected with a vaccine strain are not included in the herd infection rate, but the infected animals should be branded with a ‘S’ and sold for feeding or immediate slaughter. Each state’s brucellosis program is evaluated by a review committee at least once every three years and, under the Emergency Action Plan, all Class A states are reviewed quarterly. 17,25 A state’s or area’s status can be terminated at any time if it does not have adequate surveillance measures, has excessive herd infection rates, or fails to comply with the Uniform Methods and Rules (UM&R) for brucellosis.

a. State classifications

1) Class B. To qualify for Class B status, a state must have an annual herd infection rate of 1.5% or less, and the state must reduce the prevalence of brucellosis over a 2-year period. 17 A state with a herd infection rate of more than 1.5% but fewer that 10,000 herds is reviewed by APHIS to determine whether it qualifies for Class B status. This evaluation is based on factors such as the number of herds at risk of infection, the pattern of infection (e.g. scattered or clustered), and the effectiveness of herd management plans in the infected herds. Class B states that do not meet the minimum standards in the UM&R are placed under Federal quarantine. 17
Surveillance in a Class B state includes the brucellosis milk surveillance test (BMST), which tests bulk milk samples from dairies for brucellosis at least four times a year, and the market cattle and bison identification (MCI) program, which tests individual animals for brucellosis when they are sent through markets or to slaughter. Herds with suspicious BMST results are investigated within 30 days and undergo a herd blood test, if warranted. Within 30 days, MCI reactors must also be traced to their herd of origin, which is quarantined and tested for brucellosis. At least 80% of the MCI reactors must be traced to their herd of origin and at least 90% of the traced MCI reactor cases must be successfully closed each year in a Class B state.

Female dairy cattle and bison 4 months old or older must be official vaccinates if they are moved either into or out of a Class B state, unless they are sent directly to slaughter or to a quarantined feedlot. As of 1990, the only cattle or bison that can be moved from Class B states not meeting the standards for progress are steers, spayed heifers, and bulls less than 18 months old from unaffected herds, ‘S’-branded animals, and animals from Certified Brucellosis-Free Herds.

2) Class A. To qualify for Class A status, a state must have an annual herd infection rate of 0.25% or less. Infected herds must have a herd plan to eliminate the infection or be depopulated. A state with a herd infection rate of more than 0.25% but fewer that 10,000 herds is reviewed by APHIS to determine whether it qualifies for Class A status. This evaluation is based on factors such as the number of herds at risk of infection, the pattern of infection (e.g. scattered or clustered), and the effectiveness of herd management plans in the infected herds.

The BMSTs must be done at least four times a year in Class A states. At least 90% of the MCI reactors must be traced back to their herd of origin and at least 95% of the traced MCI cases must be successfully closed. MCI reactors must be traced within 15 days and herds with suspicious BMSTs must be investigated within 15 days. Up to two circumscribed state–federal quarantines can exist in Class A states. The state must apply for approval and the application must include a plan for placing and enforcing the quarantine(s), procedures for moving animals within and from the area, and plans for eliminating the foci of infection within the next two years. A state can retain Class A status indefinitely, as long as it continues to meet the requirements for that status. Class A states that do not meet the minimum standards in the UM&R may be reclassified to Class B.

3) Class Free. To qualify for Class Free status, a state or area must have been free of field strains of *B. abortus* in domestic cattle and bison for at least 12 months. All herds must be released from quarantine and all other domestic livestock must be free of *B. abortus* at the time of certification.

The BMSTs are done at least twice a year in Class Free states. At least 90% of MCI reactors must be traced to their herd of origin and at least 95% of the MCI reactor cases must be successfully closed. Herds with a suspicious BMST or MCI reactors must be investigated or traced, respectively, within 15 days of the test date.
A state may remain Class Free when one affected herd is found, if the herd is quarantined immediately, tested for brucellosis, and depopulated if found infected.\textsuperscript{17,25} An epidemiologic investigation must determine that the infection has not spread beyond the herd. These steps must be competed within 60 days.\textsuperscript{17,25} If two herds are found within a 2-year period, the state reverts to Class A status. A state can also remain Class Free if an infection is found in imported animals and has not spread to other herds before the exposed animals are returned to the originating state or slaughtered.\textsuperscript{17} Unless these conditions are met, Class Free status is automatically suspended if a field-strain of \textit{Brucella abortus} is found.

A state can retain its Class Free status indefinitely, as long as it continues to meet the requirements for that status.\textsuperscript{17} Class Free states that do not meet the minimum standards in the UM&R may lose their status and be reclassified as Class A.

\section*{4. CONTROL PROGRAM STATUS}

Brucellosis has been eliminated from domestic cattle herds in most states in the U.S. In 2002, Texas and Missouri were the only states in Class A status, with the remaining 48 states, Puerto Rico, and the U.S. Virgin Islands in Class Free status.\textsuperscript{14,15} Also during this time, 81.3\% of all beef and dairy cattle were found in Class Free States and 18.7\% in Class A States.\textsuperscript{15} Seventy seven percent of postparturient beef cows and 95\% of dairy cows were in Class Free States.\textsuperscript{15} Canada has been free of bovine brucellosis since 1985, but \textit{B. abortus} still exists in Mexico.

As progress continues toward eradication, APHIS is increasingly focusing on surveillance and on testing adjacent, contact, and community herds.\textsuperscript{14,15,23} Fourteen newly affected herds were found in 2000, six in 2001 and nine in 2002.\textsuperscript{14,15} Eight of the herds found in 2002 were cattle herds and were depopulated.\textsuperscript{15} A herd plan to eliminate brucellosis was established for the ninth, a herd of bison.

The management of brucellosis in Yellowstone National Park and the Greater Yellowstone Area is also a priority.\textsuperscript{15} Currently, the focus is on managing wild bison and elk to keep them from transmitting brucellosis to domestic cattle.\textsuperscript{12} Brucellosis control efforts include the vaccination of heifers and calves, test and slaughter of reactors, and hazing of animals back inside park boundaries during winter food shortages.\textsuperscript{14} In the future, APHIS expects to develop a plan to eliminate brucellosis from bison and elk in the Greater Yellowstone area, while retaining a wild, free-roaming bison herd in Yellowstone Park.\textsuperscript{12}

\section*{5. EMERGENCY ACTION PLAN (EAP)}

The original goal of the Emergency Action Plan (EAP) was to eliminate brucellosis from cattle in the United States by December 31, 1998.\textsuperscript{25} Although the EAP did not meet its deadline, its provisions remain in effect and are expected to continue until brucellosis has been eliminated.\textsuperscript{14,15}

As a result of the EAP, all brucellosis surveillance activities, including epidemiologic investigations, are given top priority.\textsuperscript{25} New cases of brucellosis are managed as emergencies
and these cases, as well as the investigation, testing, and monitoring of high risk herds, are high priority efforts.

Although the EAP does not prohibit brucellosis control by other means, depopulation has become the preferred method for handling newly diagnosed herds and exposed high risk animals and herds. 14,15,25 A minimum one-mile radius of concern is created around each new focus of brucellosis and all high risk herds should be monitored for at least a year after the last reactor is removed.25

The EAP also provides for public information campaigns and quarterly program reviews in each Class A State to ensure compliance with the eradication program. 5

6. DIAGNOSTIC TESTS

Cattle, bison, and Cervidae can be classified as reactors on the basis of official serologic tests, a significant rise in titer, or epidemiologic evidence of infection.17,26 Animals are classified as brucellosis negative if all laboratory tests that have been done are negative. Animals are classified as suspects when the serologic tests are inconclusive. The designated brucellosis epidemiologist can reclassify animals if there is epidemiologic justification.17 Brucellosis can also be confirmed by isolation of Brucella from the animal or by agglutination in the semen plasma test.17

Under the MCI program, the approved presumptive serologic tests in cattle and bison include the buffered acidified plate antigen (BAPA) test, rapid screening test (RST), and rapid automated presumptive (RAP) test.17 Animals that are positive on a presumptive test are retested with official brucellosis tests (i.e., the card test, standard plate test (SPT), tube agglutination test, the rivanol test, manual complement fixation (CF), and technicon automated complement-fixation tests). The particle concentration fluorescence immunoassay™ (PCFIA) can be used as either a presumptive or official diagnostic test.

Supplemental tests, to help differentiate reactions due to field strains of Brucella abortus, the Strain 19 vaccine, and nonspecific agglutinins, include the card test, the mercaptoethanol (ME), Coombs test, heat inactivation (HI), acidified plate antigen (APA), fluorescent antibody (FA), enzyme-labeled antibody (ELA), concentration immunoassay test (CITE®), field enzyme-labeled immunosorbent assay (ELISA), and D-Tec® competitive cELISA tests.17 A designated brucellosis epidemiologist is the only person allowed to use and interpret the results of supplemental tests.

The card test can be a presumptive test, an official test, or a supplemental test.17 It can be used for surveillance in the MCI program, on routine samples collected on farms, and in tests of suspicious and affected herds. The card test can be used as the sole official test at the owner’s request, if no other test is practical, or if it is designated as the official test for stockyards in a state.17 Animals that are positive on the card test are classified as either reactors or suspects based results from supplemental tests.17 If no other tests are done, the animal is classified as a reactor.
The semen plasma test is an official test in bulls used for artificial insemination; serum is also tested. Bulls are classified based on the maximum agglutination titer of either the blood test or the semen plasma test.

The Brucellosis milk surveillance testing (BMST) includes the standard brucellosis ring test (BRT) on pooled milk samples, the modified BRT on pooled cream samples, and a serially diluted BRT procedure. The BRT is less reliable in herds with more than a hundred cows. False-positive reactions are common, particularly when the sample contains colostrum, milk from cows vaccinated during the previous four months, or milk from cows with mastitis. The heat-inactivated ring test (HIRT) is an approved supplemental test for milk or cream, when the test results from the BRT are suspicious. A designated brucellosis epidemiologist must evaluate the HIRT results in combination with epidemiologic factors. The IDEXX HerdChek® Milk Antibody Test is an official milk ELISA test that can be done on pooled milk from dairy herds. This test is only used for samples that contain milk from fewer than 1,000 animals. It can also be used for individual animals.

The official brucellosis serologic tests in Cervidae include the card test, the standard plate agglutination test, complement fixation, and the rivanol test.

Regardless of the results of serologic tests, an animal is classified as a reactor if field strains of Brucella are recovered from the blood, milk, or tissues. B. abortus infections are assumed to be caused by a field strain until they are cultured or otherwise proven to be a vaccine strain. Brucella colonies are slow growing and fastidious, raised, convex, and usually 0.5 to 1 mm diameter. Smooth colonies are shiny and pale yellow in transmitted light, but bluish grey and slightly opalescent in reflected light. Rough colonies are similar in size and shape but are dull white or yellowish in reflected light and are more opaque. Brucella abortus Strain 19 and strain RB51 can be differentiated from field strains by laboratory tests.

a. Field
Some tests, including the standard agglutination BAPA, and card tests can be done by authorized individuals in the field, but must be submitted by that person to a cooperative State-Federal brucellosis laboratory for confirmation of the results. The state animal health official can designate the card test as the official test in stockyards; in this case, the only other official test allowed in stockyards is the BAPA but the CITE®, standard plate, and rivanol tests can be used as supplemental tests. Persons authorized to do field tests are formally evaluated once a year.

b. Laboratory
Cooperative State-Federal brucellosis laboratories perform official brucellosis testing as well as confirm the results from field tests. In addition to other tests, state and regional laboratories are equipped to do complement-fixation, rivanol tests, culture samples for determination of the number of live organisms in an approved vaccine, and isolation of Brucella from milk, blood, and tissues. State and regional laboratories that do brucellosis testing are evaluated once every three years.

1) Classification of titers. Cattle and bison are classified as negative, suspects, or reactors. In some cases, the results from several serologic tests are combined to classify an animal.
2) Buffered acidified plate antigen (BAPA) test, rapid screening test (RST) and rapid automated presumptive (RAP) test. The BAPA, RST, and RAP tests are only used to identify sera that must be tested further. They are not used to classify animals as suspects or reactors. The results of these tests are recorded as either negative, if no agglutination occurs, or positive, if there is any agglutination. Sera from vaccinated and unvaccinated cattle are not read differently.

3) Standard plate (SPT) and standard tube (STT) tests. Cattle that have not been vaccinated with Strain 19 are classified as negative if there is complete agglutination at a dilution of 1:25 or less; suspects if there is any agglutination at 1:50 or incomplete agglutination at 1:100; reactors if there is complete agglutination at 1:100 or higher dilutions. Animals vaccinated with Strain 19 are classified as negative if there is complete agglutination at a dilution of 1:50 or less; suspects if there is any agglutination at 1:100 or incomplete agglutination at 1:200; reactors if there is complete agglutination at 1:200 or higher dilutions. See Table 1.

<table>
<thead>
<tr>
<th>Test results</th>
<th>Test interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50</td>
<td>1:100</td>
</tr>
<tr>
<td>Non-Strain 19-vaccinated</td>
<td>Strain 19-vaccinated</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>1</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: - = no agglutination; 1 = incomplete agglutination; + = complete agglutination.

Animals vaccinated with the Strain 19 vaccine as adults are not classified as reactors in the SPT or STT until the titers from their vaccinations have become negative again.

4) Card test. Both vaccinated and unvaccinated cattle are classified as negative if there is no agglutination and positive if there is agglutination. Any animal that is positive on the card test must be classified as either a suspect or reactor, depending on the additional tests done.

Both vaccinated and unvaccinated cattle are classified as reactors unless they test negative on supplemental tests such as the standard plate test, rivanol test, CITE test, and other tests. If no supplemental tests are done, the animal is also considered to be a reactor.

Animals that were not vaccinated with Strain 19 are classified as suspects if the card test is positive, but the SPT or STT is negative. Strain 19 vaccinated animals are classified as suspects if the card test is positive and other tests support the classification of this animal as a suspect rather than a reactor.

5) Rivanol test. Both vaccinated and unvaccinated cattle are classified as negative if there is incomplete agglutination at the 1:25 dilution or lower. Strain 19 vaccinated animals are also classified as negative if there is incomplete agglutination at 1:50 and the animal was vaccinated as an adult within the last 5 months.
The rivanol test is not used to classify non-Strain 19 vaccinates as suspects. Animals vaccinated with Strain 19 are classified as suspects if there is complete agglutination at the 1:25 dilution or higher but no agglutination at 1:100. In addition, the CF test must have been either negative or suspect.\(^\text{17}\)

Animals that were not vaccinated with Strain 19 are classified as reactors if there is complete agglutination at the 1:25 dilution or higher and 1) the CF test was either not done or classified the animal as a reactor, or 2) incomplete agglutination occurred at 1:100 or higher dilutions.\(^\text{17}\) See Table 2.

### Table 2—Interpretation of rivanol test results

<table>
<thead>
<tr>
<th>Test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:125 or lower (but within 5 months after adult vaccination of cattle or bison, a +1.50 titer or lower is considered negative.)</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Not applicable</td>
</tr>
<tr>
<td>+1:25 to +1:50, provided the CF test is performed and is interpreted as negative or suspect, as described in paragraphs 1 and 2 in this section.</td>
</tr>
<tr>
<td>Suspect</td>
</tr>
<tr>
<td>+1:25 or higher</td>
</tr>
<tr>
<td>Reactor</td>
</tr>
</tbody>
</table>

Key: = incomplete agglutination; = complete agglutination.

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6) **Manual complement fixation (CF) test.** Cattle that have not been vaccinated with Strain 19 are classified as negative if the 1:10 dilution or lower is 1+ (25% fixation); suspects if the 1:10 dilution or higher is 2+ (50% fixation) but the 1:20 dilution is less than 2+ (50% fixation); reactors if the 1:20 dilution or higher is 2+ (50% fixation).\(^\text{17}\)

Animals vaccinated with Strain 19 are classified as negative if the 1:10 dilution or lower is 1+ (25% fixation); suspects if the 1:10 dilution is at least 2+ (50% fixation) but the 1:40 dilution is less than 1+ (25% fixation); reactors if the 1:40 dilution or higher is 1+ (25% fixation).\(^\text{17}\)

### Table 3—Interpretation of manual complement-fixation test results

<table>
<thead>
<tr>
<th>Test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+ 1:10 or lower</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>2+ 1:10 through 1:20</td>
</tr>
<tr>
<td>2+ 1:20 through 4+ 1:20</td>
</tr>
<tr>
<td>Suspect</td>
</tr>
<tr>
<td>2+ 1:20 or higher</td>
</tr>
<tr>
<td>Reactor</td>
</tr>
</tbody>
</table>

\(^1\) Includes Strain 10 adult vaccinates cattle and bison beginning 3 months after vaccination.

Key to degree of fixation of complement: 1+ = 25 percent, 2+ = 50 percent, 3+ = 75 percent, 4+ = 100 percent.
Table 5 summarized the serologic classifications of cattle and/or bison by vaccination status.
7. IMPACT OF VACCINATION ON ERADICATION EFFORTS

Vaccination is an important part of the brucellosis eradication program. Two vaccines - Strain 19 and RB51 - provide good immunity to moderate challenge by field strains.\(^2\)

Strain 19 is a live vaccine which, given to sexually immature cattle, acts as an attenuated strain.\(^2\) It is approximately 65\% effective in preventing infections under field conditions.\(^{12}\) Some animals vaccinated with Strain 19 develop persistent antibody titers that interfere with some serologic tests.\(^2\) Rarely, localized infections with Strain 19 occur in the genital tract.\(^2\) Strain 19 can also cause arthropathy, particularly of the femorotibial joints, as well as abortions in pregnant cows.\(^2\) Reversion to virulence is extremely rare, but can occur if this vaccine is accidentally given to pregnant animals.\(^2\) Although the USDA has not removed Strain 19 from the market or the Brucellosis Eradication Program, it is no longer produced and some states prohibit its use.\(^{27}\)

Strain 19 has been replaced in the U.S. by the RB51 vaccine. RB51 is licensed for use in cattle and approved for use in the Cooperative State-Federal Brucellosis Eradication Program.\(^{27}\) This vaccine is attenuated in both calves and adults and does not induce antibodies that react in routine brucellosis tests.\(^2\) RB51 is much less likely than Strain 19 to cause abortions, although a few abortions have been seen in the field.\(^2,^{27}\) RB51 is labeled to be given to 4 to 12 month old calves.\(^2,^{17,22,27}\) Cattle over a year of age can be vaccinated if authorized by state or federal animal health officials.\(^{17}\) In preliminary studies, the RB51 vaccine has been safe and effective in bison calves, but additional studies are needed.\(^{12,27}\)

a. Official calfhood vaccinates
Official calfhood vaccinates are female cattle or bison vaccinated, when they are 4 to 12 months old, by an accredited veterinarian or a state or federal animal health representative.\(^{17,22}\) Official calfhood vaccinates are permanently identified by a tattoo and an official vaccination eartag in the right ear. An individual animal registered breed association registration brand or tattoo can be used in place of the official eartag. The brucellosis tattoo includes the U.S. Registered Shield and a ‘V’, preceded by the quarter of the year for animals vaccinated with Strain 19, or an ‘R’ for animals vaccinated with RB51. In both cases, the number at the end of the tattoo is the last digit of the year the animal was vaccinated.

b. Adult vaccinates
Cows or female bison older than a year can be vaccinated by an accredited veterinarian or federal or state representative, with approval from the state animal health official and the Area Veterinarian in Charge.\(^{17,22}\) Animals must test negative for brucellosis within 10 days before they are vaccinated. All adult vaccinates are permanently identified with a ‘V’ brand on the hip near the tailhead or an official adult vaccination (AV) tattoo in the right ear. The animal must also be identified by an official eartag or an individual animal registered breed association registration brand or tattoo.

c. The future of vaccination
Brucellosis vaccination is still an important part of the eradication program in many states, but is declining in the U.S. overall.\(^{15}\) In areas where brucellosis has been eradicated, surveillance is adequate, and reservoirs do not exist in wildlife, vaccination may not be necessary. Approximately 4.7 million calves were vaccinated in 2001 and 4.4 million in 2002.\(^{15}\)
Before the U.S. can be declared free of brucellosis by the OIE, vaccination will have to be stopped for three years. Vaccination will only be phased out after brucellosis has been completely eradicated. A working group has been formed to define the steps necessary to phase out RB51 and Strain 19 or other animal vaccines.

8. ROLE OF VMO IN BRUCELLOSIS ERADICATION PROGRAM

a. Testing and diagnosis
Two official programs for brucellosis surveillance – the MCI program and the BMST program – exist for cattle and bison. Under the MCI program, animals are blood tested for brucellosis at slaughter and when they are sent to markets or other points of concentration. Test-eligible animals include sexually intact cattle and bison 18 months old and older, and all parturient and postparturient animals, regardless of age and vaccination status. Steers and spayed heifers are not tested. Dairy cattle vaccinated with Strain 19 are exempt until they are 20 months old and beef breeds or bison vaccinated with Strain 19 are exempt until they are 24 months old. If an animal is classified as an MCI reactor, it is traced to its herd of origin and the herd undergoes a complete herd blood test.

The BMST program monitors the nation’s milk supply to detect infected dairy herds. Pooled milk or cream from dairy herds is tested 2-4 times a year and herds with suspicious results receive a complete herd blood test.

A herd blood test (HBT) includes all animals six months old or older, except bulls less than 18 months old, spayed heifers, and steers. Dairy cattle vaccinated with Strain 19 are exempt until they are 20 months old; Strain 19 vaccinated beef cattle or bison are exempt until they are 24 months old. Parturient or postparturient animals are included in the herd test regardless of their vaccination status and age. The owner of the herd may request a retest, at his or her own expense, within three days after being notified of the results.

In addition to MCI and BMST testing, animals are tested when they change ownership or are exhibited at shows. Rodeo bulls participating in events can be moved without testing, if they have had a negative brucellosis test within the last year, are individually identified, and are not sold or transferred to a new owner.

Test-eligible animals from Class A and B states must have a negative brucellosis test within the 30 days before they are moved to another state, or be tested on arrival at an approved stockyard. This requirement is waived in certain cases when animals do not change ownership, to allow ranchers to carry out normal farming practices. Test-eligible animals from Class Free states and Certified Brucellosis-Free Herds can be sent interstate for breeding, without a brucellosis test, if their herd of origin is identified. Animals from all non-quarantined herds can be sent directly to slaughter without pre-movement testing if their herd of origin is identified. Cattle and bison exempt from testing can move interstate or intrastate without a certificate, unless they are from a quarantined herd.

1) Certified brucellosis free cattle and bison herds. A herd of cattle or bison can qualify as a Certified Brucellosis-Free herd with four consecutive negative BMSTs, at intervals of at least 90 days, followed by a negative herd blood test within the next 90 days. A herd can
also qualify with at least two consecutive negative herd blood tests 10-14 months apart. The herd’s status includes all calves. To remain certified, the herd must have a single annual negative herd blood test. Animals from Certified Brucellosis-Free herds are exempt from brucellosis testing when they are moved for breeding or sold.17,22

Breeding animals can be added to the herd from a Class Free state or another Certified Brucellosis-Free Herd without testing.17 Animals from other herds in Class A or B states cannot come from quarantined herds or feedlots and must have a negative blood test within 30 days before transfer. All animals must have a blood test 60-120 days after being added to the herd. Certified Brucellosis-Free herds maintain their certification if they are bought and remain on the same premises or are moved to a new site that has no other cattle or bison.17

If reactors are found in a Certified Brucellosis-Free herd, the herd is quarantined and tested.17 Certification is reinstated if the reactors are infected with vaccine strains. A herd infected with a field strain can only re-qualify if it is released from quarantine and also meets the provisions for initial Certified Brucellosis-Free herd status.

2) Qualified herds. Qualified herds are cattle or bison herds, located in a quarantined area, that are negative on two consecutive herd tests.22 The first herd test must take place 120-240 days before the herd is certified as a qualified herd. The second herd test is done 90-150 days after the first test. Certified Brucellosis-Free herds are also considered to be qualified herds if they are negative on a herd blood test 120 days before or after the quarantine was placed.22 To remain qualified, a herd must be retested within 120 days of the last herd test.

3) Certified brucellosis free cervid herds. Certified Brucellosis-Free cervid herds qualify with three consecutive negative tests, with each test 9-15 months apart.26 All sexually intact animals that are at least six months old must be included in the tests. Certification is good for 24 months and herds are recertified with a single negative herd test. Unless they come from another Certified Brucellosis-Free herd, replacement animals are not considered part of the herd until they have passed three blood tests - the first during the 30 days before shipment from the herd of origin, the second 60-180 days after being added to the herd, and the third as part of the herd re-certification test.26 Like cattle herds, Certified Brucellosis-Free cervid herds maintain their certification if they are bought and remain on the same premises or are moved to a site that has no other animals.26

4) Brucellosis monitored cervid herds. A Brucellosis Monitored cervid herd is a herd, raised on the range, that is statistically likely to be brucellosis-free. The initial herd test must include enough six month old and older, sexually intact animals to have a 95% probability of detecting a brucellosis prevalence of 2% in the herd.26 The herd receives monitored status for 12 months, and is re-certified by testing 25% of the initial sample size. Over each three year period, 100% of the initial sample size must be tested. Unless an animal comes from a Certified Brucellosis-Free cervid herd, it must be tested for brucellosis before it is added to a Monitored Herd.26 Animals from other Monitored Herds are tested once, within the 90 days before transfer. Animals from other herds must have two negative tests, 90 days apart, before transfer, and another test 90 days or more after being added.
b. Collection and submission of samples

Pooled milk samples are tested at least four times a year from milk receiving stations, dairy processing plants or individual dairy herds in Class A and B states and at least twice a year in Class Free states. Samples are collected from pipeline segments, bulk tanks, or milk cans. All commercial dairies must be included in at least three tests each year in Class A and B states and at least two tests in Class Free states. Fresh milk samples should be used for the BRT, if the sample contains milk from 150 or more cows, but formalin- or potassium dichromate-preserved samples can be used for herds with less than 150 cattle. The same procedures are used to collect samples for the IDEXX HerdChek® Milk Antibody Test (milk ELISA). The milk ELISA is done on pooled milk from fewer than 1,000 animals, or as a test for individual animals.

The serially diluted BRT is done on individual cows, either on separate milk samples from individual quarters or on a composite sample that contains equal quantities of milk from each quarter.

Serum samples are collected from test-eligible animals at slaughter and when they are sold, sent to market, or moved to another state. Animals tested under the MCI program procedures should have an official eartag and/or a USDA-approved backtag, to identify the herd of origin. All tags must be collected at the time of slaughter and accompany the animal’s blood sample to the laboratory. Tattoos and brands are not collected.

c. Managing an infected herd

An infected herd must be managed under quarantine until it has been depopulated or is free of brucellosis. All animals except steers and spayed heifers are included in the quarantine. Reactors are tagged in the left ear, branded with a ‘B’ brand on the left hip near the tailhead, and sent to slaughter. All exposed premises, equipment, and vehicles are decontaminated. Until the quarantine is lifted, exposed animals can be moved only to immediate slaughter, a quarantined feedlot, or a quarantined pasture. Exposed animals that leave the herd must be ‘S’-branded, unless they are shipped directly to slaughter in an officially sealed truck or are finished-fed heifers being sent directly to slaughter. Animals classified as suspects on serologic tests are treated like exposed animals.

All herds that have sold animals to, received animals from, or been in contact with the infected herd are traced and tested for brucellosis. These herds must have an approved herd plan within 15 days of locating the herd of origin or the recipient herd in a Class A or Class Free state, and within 45 days in a Class B state.

A state can retain its Class Free status if it has one infected herd and the herd is quarantined immediately, tested, and depopulated. Brucellosis must not have spread beyond the herd and the depopulation and epidemiologic investigation must be completed within 60 days.

All herd owners in the immediate community must be notified of a quarantined herd within 30 days and the state public health agency must be notified within 15 days.

1) The herd plan. The herd owner, his or her veterinarian (upon the owner’s request), and a veterinarian of the Brucellosis Program develop a formal, written herd plan to eliminate the infection. The purpose of the plan is to eliminate brucellosis from the herd, prevent its reintroduction, and prevent it from spreading to other herds. Under the Emergency
Action Plan, depopulation is the preferred method for handling an infected herd. Class Free states must depopulate infected herds to retain their status.

Factors to be considered in a herd plan include:

- The brucellosis classification of the state
- The risk that brucellosis will spread to other herds
- The infection and potential exposure rates within the herd
- The type of cattle and/or bison operation
- Herd management practices and economic factors that relate to brucellosis control

The individual herd plan should include:

- Testing schedules
- Herd health management practices
- Procedures to handle preparturient and parturient cows
- Procedures for adding animals to, or removing them from, the herd
- Identification of animals
- Sanitation practices
- Vaccination plans. All heifer calves kept in the herd should be vaccinated.

2) Whole herd vaccination plan. Under a Whole-Herd Vaccination Plan (Adult Vaccination), the reactors are removed and all remaining animals are tested for brucellosis. Female cattle and bison that test negative are vaccinated within 10 days of the test results.

After vaccination, a herd blood test is done, either in two months or as soon as possible, to help eliminate any remaining infections. Test and slaughter procedures must be re-started within 6 months after the vaccination. Tests are done at intervals specified by the individual herd plan. For the first four years after the whole-herd vaccination, animals added to the herd can be vaccinated as adults if they have a negative test within 10 days before entering the herd.

Whole-herd vaccination can also be done on uninfected herds if they are at a high risk of becoming infected and the owner receives written approval from state and federal officials. An individual herd plan, including procedures to prevent the introduction of brucellosis into the herd and control its spread, must be developed and agreed upon.

3) Quarantine release. An infected herd is released from quarantine if it has two consecutive negative herd blood tests, 30 and 180 days after all reactors have been removed. The herd test for quarantine release includes all non-neutered animals that are at least six months old. All heifer calves vaccinated with Strain 19 at least six months ago are included. The quarantine release test establishes baseline titers for each animal, which makes changes in titer at the post-quarantine retest easier to evaluate.

A post-quarantine test is done on all non-neutered animals that are over six months old, either 6-12 months after the herd has been released from quarantine or 10-16 months after the last reactor was removed.

Beef cattle and bison participating in a whole-herd vaccination plan ("AV" herds) are released from quarantine with the usual quarantine release and post-quarantine tests. "AV" dairy herds must fulfill these requirements and also be negative on the last herd BMST. If an "AV" dairy
herd is released from quarantine but is suspicious on the herd BMST, it is evaluated by the designated brucellosis epidemiologist and monitored as needed.

4) Animals in quarantined feedlots. The purpose of a quarantined feedlot is to finish-feed cattle or bison in drylot. All animals in a quarantined feedlot are classified as brucellosis-exposed and are intended for slaughter. In Class A and Class B states, test-eligible cattle and bison must be tested either when they enter or during the previous 30 days. Negative exposed and untested test-eligible animals must be ‘S’ branded on entry. All animals that leave a quarantined feedlot, other than steers and spayed heifers, can be sent only to slaughter or to another quarantined feedlot. Animals may be sent either directly or through an approved intermediate handling facility. Except for spayed heifers and steers, animals from a quarantined feedlot must be ‘S’ branded if they are not sent directly to slaughter.

Quarantined feedlots are discouraged in Class Free states, but may be allowed if brucellosis suspects or exposed animals are not being sent directly to slaughter. In Class Free states, animals entering the feedlot do not have to be tested. These feedlots cannot receive animals from Class A or B states. Approval expires in one year.

5) Approved bison quarantine facilities. Approved bison quarantine facilities (ABQFs) test bison from Yellowstone and Grand Teton National Parks, to qualify them as brucellosis-free. Animals must test negative to enter an ABQF. These animals are considered to be brucellosis-exposed and are permanently identified with official metal eartags.

In the ABQF, bison are placed in individual test groups (ITGs), and periodically retested for brucellosis. Animals are only released from quarantine when all of the bison in their ITG qualify. To be released, sexually mature male bison (three years old or older) must be negative on three consecutive ITG tests. The first test is done at the start of the quarantine period, the second after at least 180 days, and the third a year or more after the first test. The same rules apply to sexually immature male bison, but the last test must be done when they are at least three years old. If any reactors are found, the ITG must re-start the quarantine and be tested every 30-45 days until all reactors have been removed and a complete ITG test is negative.

Female bison that enter the ABQF must complete at least one calving before release. During each calving, an ITG test is done 30-90 days after each female has calved, and six months after the last animal in the group has calved. There must be at least 12 months between the first and last consecutive negative ITG test. Discharges, fluids, and swabs are cultured from each animal within five days after it has given birth. Nonpregnant mature and immature females must be bred to a test-negative male and complete one calving in the facility. Female bison that were pregnant before entry must complete a second calving after being bred to a test-negative male.

Calves conceived before their dam entered the ABQF can only qualify for release if they test negative under the same schedule as immature bison. Calves born to dams bred in the ABQF can be released once they are at least six months old, if no reactors were found in the ITG around their time of birth and all other calves in the ITG are brucellosis negative. Calves born during a period when reactors are found are classified as immature bison and must pass the same tests as other immature animals.
Neutered bison can be released from the ABQF without restrictions at any time. Unless they qualify for quarantine release, other animals can be sent only to an approved research facility or to slaughter. All reactors and culture-positive animals must be removed from the ABQF within 15 days.

Bison that have been released from quarantine must be retested approximately 6 and 12 months after release, and must be kept separate from all other animals until the six month test is negative.

d. Surveillance
The goals of surveillance are to find the last cases of brucellosis in domestic animals, measure the progress and efficacy of the eradication program, demonstrate the low prevalence or absence of brucellosis for international trade, and rapidly detect brucellosis if it is introduced from other countries.

Primary surveillance methods include the MCI program, BMSTs, and large scale area testing. Routine surveillance also occurs during change-of-ownership testing, when animals are moved interstate, and when shows or exhibitions require brucellosis tests. Annual herd tests on Certified Brucellosis-Free herds provide sentinel surveillance.

Secondary surveillance includes those procedures that occur after an infected herd has been found, including epidemiological tracebacks, area testing, and diagnostic testing.

Of the nine infected herds identified in 2002, five were found in the MCI program and one during a post-quarantine release test. One herd was discovered when it developed brucellosis symptoms, one herd was adjacent to another infected herd, and one herd was found during an epidemiologic investigation.

9. COMPARISON OF SURVEILLANCE METHODS

a. Market cattle and bison identification (MCI) program
The MCI program tests cattle and bison sent to slaughter or moved through markets and stockyards. Market testing has been very useful in finding infected herds in states that are Class A or have recently become Class Free. In 2002, 10.5 million cattle were tested for brucellosis. The vast majority of these cattle, 91%, were tested under the MCI program.

All state- or federally-inspected abattoirs that slaughter cattle or bison must participate in the MCI program and each slaughterhouse must test at least 95% of all cows and bulls that are at least two years old. At markets, sexually intact cattle and bison 18 months old and older are tested. Dairy cattle vaccinated with Strain 19 are exempt until they are 20 months old and beef breeds or bison vaccinated with Strain 19 are exempt until they are 24 months old. All parturient and postparturient animals are tested, regardless of age and vaccination status. To facilitate tracebacks, animals must be identified with an official eartag or backtag either at or before the first establishment they reach. In 2002, approximately 62% of the MCI samples were collected at slaughterhouses and 38% at stockyards.
Under the MCI program, the approved presumptive tests for cattle and bison include the BAPA, RST, and RAP tests. Animals that are positive on the presumptive test are retested with the card test, SPT, tube agglutination test, or other official tests. If the animals are not tested further, all animals that are positive on the card test, SPT, or tube agglutination test are reported as MCI reactors.

At least 90% of the MCI reactors must be traced back to their herds of origin in Class A and Class Free states and 95% of these traced cases must be successfully closed. In Class B states, 80% of tracebacks must be successful and 90% of the traced cases must be successfully closed. A case is considered to be successfully closed if the herd is blood tested and quarantined if necessary, or a herd test is found to be unnecessary. If all test-eligible animals in the herd have been slaughtered, an epidemiologic investigation should identify the source of the infection and any spread. If a reactor can be traced only to a dealer or commission firm, the traceback is not successful unless all possible herds of origin are found and tested. A state that has 20 or fewer reactors and cannot trace at least 90% (80% in class B states) can request an exemption in the annual report.

b. Brucellosis milk surveillance test (BMST)
Dairy herds are evaluated with the brucellosis milk surveillance tests at least four times a year in Class A and B states, and at least twice a year in Class Free states. All herds that produce commercial milk must be included in at least three of these tests in Class A and B states and two tests in Class Free states.

The BMSTs include the standard brucellosis ring test on pooled milk samples and the modified BRT on pooled cream samples. The heat-inactivated ring test is an approved supplemental test for milk or cream, when the test results from the BRT are suspicious. The IDEXX HerdChek® Milk Antibody Test is an official milk ELISA test that can be done on pooled milk from dairy herds. It is only used for samples that contain milk from fewer than 1,000 animals.

The test results from the BMSTs are reported as either negative or suspicious. If the test is suspicious, a herd blood test determines whether the herd is infected. This investigation must take place within 30 days in Class B states and 15 days in Class A and Class Free states.

From 1975 to 1985, the BMST found an average of 225 infected herds each year, in approximately 415 annual tracebacks. In 2002, 73 suspicious BRT results were reported and 60 herds were blood tested. None of these herds were found to be infected.

c. Cervid surveillance identification (CSI) program
The Cervid Surveillance Identification (CSI) program tests all 6-month old and older Cervidae for brucellosis before slaughter at state or USDA-approved abattoirs and when they are sent to auction markets or moved across state lines. Efforts are also made to test animals slaughtered in the field or at home. CSI reactors are traced back to the herd of origin, which is quarantined if it appears to be infected.
10. TESTING

In Class A and B states, test-eligible animals shipped interstate for breeding or other purposes must have a negative brucellosis test within the 30 days before they are moved. Alternatively, they can be tested on arrival at an approved stockyard. Animals are also tested when they change ownership and when they are exhibited at shows and exhibitions.

Owners are encouraged to test their animals 45-120 days after purchase to identify animals that are incubating brucellosis at the time of their sale. A blood test is also recommended 45-120 days after animals have been sent for breeding.

Infected animals may also be found when they develop brucellosis symptoms. Suspected cases of brucellosis must be reported by producers, veterinarians, animal health inspectors, and diagnostic laboratories.

Certified Brucellosis Free herds can serve as sentinel herds, when they are tested for their annual re-certification.

a. Community

Under the Emergency Action Plan, a minimum one-mile radius of concern is created around each new focus of brucellosis. During an outbreak, blood samples are collected “on farm” from targeted animals in a defined area. Individual states may also require area testing for brucellosis surveillance, even if no outbreaks are occurring.

b. Epidemiology

When an infected herd is identified, epidemiologic investigations are conducted to find other infected herds. Herds that should be identified include those that may have transmitted the infection, as well as herds that have received animals from or been in contact with the affected herd. Once these herds are found, they must have an approved herd plan within 15 days in Class A and Class Free states and within 45 days in Class B states. In a Class Free state, these herds are also placed under quarantine and must have a complete herd test unless the designated brucellosis epidemiologist decides this test is unnecessary.

c. Adjacent

When an infected herd is found, adjacent herds are tested for brucellosis. Adjacent herds include, at a minimum, all herds within one mile of the fenceline of the affected property. Adjacent herds must have an approved herd plan within 15 days in Class A and Class Free states and within 45 days in Class B states. Adjacent herds in Class Free states are also placed under quarantine and must have at least two complete herd tests, with the second test approximately six months after the infected herd is depopulated or released from quarantine. The designated brucellosis epidemiologist may waive the herd test if warranted.

d. Following class free status

The MCI and BMST programs continue in Class Free states, although the frequency of the BMST may be reduced to two annual tests, with all commercial herds included in each test. At least 90% of MCI reactors must be traced to their herd of origin and 95% of the reactor cases must be successfully closed.
Test-eligible animals from Class Free states are exempt when they are sent interstate for breeding, although a brucellosis test is strongly recommended 45-120 days after movement. 17

Following the identification of an infected herd in a Class Free state, a complete epidemiologic investigation must take place within 60 days, to determine whether any other herds have been infected. 17,22 All adjacent herds, source herds, and contact herds are quarantined and must have a complete herd test. Adjacent herds are also retested six months after the infected herd was released from quarantine or depopulated.

11. References


