

Update on *Leptospira hardjo-bovis* Control in Beef Herds

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Abstract

Leptospira hardjo bovis has emerged as the most common leptospiral infection of cattle in the United States and Canada. Its new name is *Leptospira borgpetersenii* serovar Hardjo (hardjo bovis), referred to as 'hardjo-bovis'. The main reason for the widespread prevalence of hardjo-bovis is that it is very efficiently transmitted by a chronic renal carrier/shedder state lasting a year or more. Also, traditional 5-way leptospira vaccines have provided poor protection against hardjo-bovis because they contain a different hardjo organism, *L. hardjo prajitno*, which is not found in North America.

Experimental and natural infections of cattle with hardjo-bovis result in reproductive losses including embryonic death, abortion, stillbirths and weak calves. In addition, involvement of the mammary glands in hardjo-bovis infections can result in the "mastitis/flabby bag syndrome" of beef cattle. In the field, it has been extremely difficult to diagnose hardjo-bovis because traditional use of serology to diagnose leptospiral infections works very poorly for diagnosis of hardjo-bovis. As the maintenance host for hardjo-bovis, cattle mount a weak and transitory antibody response to infection. Identification of leptospire in urine correlated with antibody titers to hardjo-bovis and several other serovars of leptospira can overcome that obstacle to diagnosis. The combination of losses typically not noticed by ranchers, such as embryonic deaths, and the difficulties in diagnosing hardjo-bovis as the cause of obvious losses like abortions have made hardjo-bovis a "hidden disease".

Programs to control hardjo-bovis in beef herds are now possible because of the identification of two antibiotics that can clear the renal carrier state of hardjo-bovis and the availability of new leptospira vaccines that contain hardjo-bovis. Hardjo-bovis control programs have four parts: 1) enhancement of general herd resistance; 2) biosecurity; 3) antibiotic treatment to eliminate the renal carrier state; and 4) vaccination. The primary consideration in the decision on whether to implement a control program for hardjo-bovis in a beef herd is *how great is the disease threat?*

There are still gaps in our understanding of hardjo-bovis infection of beef herds. Diagnostic testing is still cumbersome. Hardjo-bovis-specific poly-

merase chain reaction (PCR) tests now being perfected will make possible accurate, fast diagnoses of hardjo-bovis in urine or tissue samples. That will greatly aid in identification of problem herds and in monitoring the success of control programs. Like most infectious diseases of cattle, clinical trials are needed in United States beef cow/calf operations to determine the herd-level economic impact of hardjo-bovis infection and efficacy of control programs.

Résumé

La leptospirose due à *Leptospira hardjo-bovis* est devenue l'infection leptospirale la plus commune chez les bovins des États-Unis et du Canada. L'agent causal porte un nouveau nom, *Leptospira borgpetersenii* sérovar Hardjo (*hardjo bovis*), mais on le nomme aussi communément « hardjo-bovis ». La principale cause de la grande prévalence du hardjo-bovis, c'est sa transmission très rapide par les bovins qui deviennent porteurs/excréteurs au niveau des reins, un état qui dure un an ou plus. D'autre part, les vaccins pentavalents traditionnels se sont avérés peu efficaces contre le hardjo-bovis parce qu'ils contiennent un microorganisme différent, *L. hardjo prajitno*, qui n'existe pas en Amérique du Nord.

L'infection expérimentale ou naturelle avec le hardjo-bovis provoque chez les bovins des pertes au niveau de la reproduction : mort embryonnaire, avortement, mort à la naissance, veaux faibles. De plus, l'atteinte des glandes mammaires lors de l'infection au hardjo-bovis peut se traduire par le syndrome « mammite/pis flasque » des bovins de boucherie. Sur le terrain, il est extrêmement difficile de diagnostiquer l'infection par le hardjo-bovis avec la sérologie de diagnostic traditionnelle des leptospiroses, qui s'avère très peu efficace. Il est vrai que les bovins porteurs du hardjo-bovis développent une réaction immunitaire faible et transitoire à l'infection. Toutefois, on peut surmonter cet obstacle grâce à l'identification des leptospire dans l'urine, corrélée au titrage des anticorps au hardjo-bovis et à d'autres sérovars de leptospire. Étant donné les pertes comme la mortalité embryonnaire, non remarquée par les producteurs, et la difficulté d'associer le hardjo-bovis à des pertes évidentes comme les

avortements, cette maladie est en quelque sorte « une maladie cachée ».

Mais il est maintenant possible d'implanter des programmes de lutte au hardjo-bovis. En effet, on a mis au point deux antibiotiques pouvant neutraliser l'état de porteur du hardjo-bovis au niveau des reins, ainsi que des vaccins à base de hardjo-bovis. Les programmes de lutte au hardjo-bovis comportent quatre volets : 1) l'amélioration de la résistance générale des troupeaux, 2) la biosécurité, 3) le traitement antibiotique contre l'état de porteur au niveau des reins et 4) la vaccination. La question à se poser avant d'instaurer un programme de lutte au hardjo-bovis dans un troupeau de bovins de boucherie est : « *È quel point cette maladie est-elle menaçante ?* »

Nous ne comprenons pas encore tout de l'infection des bovins de boucherie par le hardjo-bovis. L'application des tests de diagnostic est encore difficile. Cependant, un test de diagnostic utilisant la réaction d'amplification en chaîne par polymérase (PCR) spécifique au hardjo-bovis, en phase de perfectionnement, permettra la détection rapide et précise de cet agent pathogène dans l'urine ou les tissus. Ce test facilitera beaucoup l'identification des troupeaux à problèmes et le suivi de l'efficacité des programmes de lutte. Comme pour la plupart des maladies infectieuses des bovins, il est nécessaire de conduire des tests cliniques dans les élevages de veaux d'embouche (« vache-veau ») des États-Unis pour déterminer l'impact économique de l'infection au hardjo-bovis dans les troupeaux et l'efficacité des programmes de lutte.

Introduction

I have followed the interesting saga of *Leptospira* hardjo-bovis for the past 30 years. An article by Hunter of Astoria, Oregon that appeared in the July 1975 issue of *Veterinary Medicine / Small Animal Clinician* first concerned me about possible reproductive losses from this pathogen in dairy herds that I served in my mixed veterinary practice in Northern California.¹⁹ Hunter observed substantial titers against *L. hardjo* in a group of unvaccinated Jersey cows that experienced several abortions and a mummified fetus. Later, I learned that some of my dairy practitioner friends were concerned enough about reproductive losses in their dairy clients' herds that they advised vaccination against *L. hardjo* with the 5-way leptospira vaccine four times a year.

Over subsequent years, I was not able to diagnose *L. hardjo* as a cause of abortion in beef or dairy cows in my practice. Laboratory results did, however, confirm diagnoses of abortion due to *L. pomona* in a client's beef herd and *L. autumnalis* in a client's dairy herd. It was only later that I learned that use of serology in the dam and fluorescent antibody tests on fetal tissues were com-

monly fruitless in diagnosis of *L. hardjo*. In 1993, Kirkbride made the following statement on diagnosis of leptospiral infections of cattle: "Methods to diagnose leptospirosis leave something to be desired".²³ Eleven years later the situation had improved very little. In 2004, Anderson stated "Establishing a diagnosis of leptospira abortion is difficult."²³ In my practice, *L. hardjo* remained a mystery organism and any disease caused by it in my clients' herds remained unknown to me.

Since then, *L. hardjo* infection has been implicated as the most common leptospiral infection in North American cattle.^{2,8,15,20-22,29,36,42} Its new proper name is *Leptospira borgpetersenii* serovar Hardjo (hardjo-bovis), hereafter referred to as 'hardjo-bovis'. In 1989, studies by Bolin showed that while 5-way leptospira vaccines have provided moderate protection against most leptospira serovars, they give minimal protection against hardjo-bovis.^{9,10} They do not contain that serovar of hardjo! They contain *L. interrogans* serovar Hardjo (hardjoprajtino) which is present in Europe, not North America, and gives little cross protection against hardjo-bovis. Ineffective control programs have allowed hardjo-bovis to become widespread in cattle of North America.

Interest in control of hardjo-bovis infection remained lukewarm for many years: it was a "hidden disease" and an effective prevention program was not available. Advances in our understanding of hardjo-bovis infection of cattle were needed. Breakthroughs came in 1999 when an epidemiologic study on a California dairy uncovered early embryonic death as possibly the most common outcome of hardjo-bovis infection,¹⁶ in 2001 when antibiotics approved for use in food animals were shown to do a pretty good job of clearing the renal carrier state of hardjo-bovis,¹ and in 2001 when a new hardjo-bovis vaccine was proven to prevent renal colonization and urinary shedding of hardjo-bovis.⁵

The potential for using these advances to successfully control hardjo-bovis infection in cattle has inspired new interest in an old disease. This update has been written to discuss new understandings of hardjo-bovis infection that have developed since BonDurant¹¹ and Hairgrove's¹⁷ presentations at the 37th Annual Convention of the American Association of Bovine Practitioners Meeting in 2004. Old understandings are discussed along with the new so that veterinary practitioners can be familiar with how widespread infection with hardjo-bovis is in cattle herds, how it's transmitted, the type and extent of resultant reproductive losses, how to diagnose infection, and how to implement effective control programs.

Prevalence Studies

The prevalence of infection of beef cattle in North America with *Leptospira* serovars has been estimated

by serologic surveys, by culture of leptospira from the kidney or urine and by identification of leptospira in urine by polymerase chain reaction (PCR). Until recently, culture, however, had an advantage over PCR for identifying specific serovars of leptospira. New serovar-specific PCR tests have eliminated that advantage.

A 2003 prevalence study conducted by the University of Texas Medical Branch at Galveston and the Texas Veterinary Medical Diagnostic Laboratory at College Station identified non-serovar specific leptospiral DNA by PCR in 106 of 300 (35%) urine samples collected from 15 to 20-month calves at slaughter.⁴³ The authors felt their study underestimated the percentage of calves with leptospiuria, because their PCR test didn't have all primers for the various strains of leptospira serovars. This study showed that over one out of three calves in a Texas feedlot were shedding leptospiral organisms in their urine at slaughter, but did not identify which serovars of leptospira were involved.

Hardjo-bovis was identified in 88% of 51 leptospira isolates from 226 kidneys cultured at a Florida slaughterhouse.⁴⁷ In Quebec, 77% of 35 leptospira isolates from 122 kidneys cultured at slaughter were identified as hardjo-bovis.¹⁵ In 1991, leptospira was isolated from 88 of 5,142 kidneys cultured from cattle at slaughterhouses located in 49 states and Puerto Rico, with 83% of isolates identified as hardjo-bovis.²⁹ Culture for leptospira in urine of cattle routinely yields a much lower percentage of positives than PCR tests because organisms present in urine sometimes fail to grow in laboratory media, either due to inhibiting factors in urine or because the organisms died during transport. Thus, hardjo-bovis is the culprit over 80% of the time in leptospiral kidney infections of cows or calves in North America.

A hardjo-bovis beef herd prevalence study conducted in Ontario, Canada found that 44% of 52 beef herds that had not been vaccinated against leptospirosis contained cows serologically positive for hardjo-bovis.³⁶ Hardjo-bovis dairy herd prevalence studies have been conducted in the US and Canada. In 2001, a hardjo-bovis herd prevalence study was conducted in 44 US dairies located in four geographic regions.⁴ Infected herds had one or more cows shedding leptospores in their urine, plus patterns of serum titers to five leptospira serovars compatible with infection by hardjo-bovis. The herd infection rate was 55% in the Florida milk-shed, 27% in the midwest milk-shed, 91% in the California Central Valley milk-shed and 55% in the northwest milk-shed. The overall US dairy herd hardjo-bovis infection rate was 57%. In Ontario, Canada, 8% of 296 dairy herds that had not been vaccinated against leptospirosis contained cows serologically positive for hardjo-bovis.³⁶

These prevalence studies indicate that hardjo-bovis

has become the dominant leptospiral infection of beef and dairy cattle in North America. A substantial percentage of slaughtered cattle have leptospiral organisms in their kidneys or urine, and the leptospiral serovar present is usually hardjo-bovis. In addition, up to half of beef and dairy herds contain cows infected with hardjo-bovis. The widespread occurrence of this organism in cattle in the US and Canada is related to its high efficiency of transmission.

Transmission

For each type of leptospira there is an incidental (accidental) host and a maintenance (reservoir) host.⁷ For all the non-hardjo-bovis leptospiras, cattle are incidental hosts and the maintenance hosts are wildlife (*L. grippityphosa*, *L. icterohemorrhagiae*), dogs (*L. canicola*) or swine (*L. pomona*). Infection in incidental hosts is severe, followed by short-term urinary shedding of organisms. Typical clinical signs include fever, anorexia, hemolytic anemia, hemoglobinuria, jaundice, uremia, abortion storms, and, in some cases, death.

Infection of maintenance hosts presents a much more subtle clinical picture. Pathogenicity is low in maintenance hosts, which usually show minimal clinical signs and mount a very weak immune response to infection. Cattle are the maintenance host for hardjo-bovis. Following infection with hardjo-bovis, cows become chronic renal carriers. Urine shedding is the heaviest during the first four months of infection, but can persist for a year or more.²⁵ Urine shedding can become intermittent as the infection progresses. Leptospiral organisms grow well in warm, moist conditions, and can survive in pond water for six months.^{14,33}

Urine is the main source of hardjo-bovis organisms. Purchase of a renal carrier animal is the most common way a herd becomes infected. Aborted fetuses and uterine discharges also contain organisms. The semen of an infected bull can contain leptospores, and some researchers feel venereal spread of hardjo-bovis is common. *In utero* exposure may also be an important route of transmission. Leptospores were detected in the kidneys of 11 apparently healthy calves born to heifers that had been experimentally exposed to hardjo-bovis during the fourth to sixth month of gestation.¹⁰

Leptospira usually enter the body through damaged or lacerated skin. Exposure of the nasal mucosa, conjunctiva or vagina to organisms results in infection because the organisms can penetrate intact mucous membranes. Deposition of only 100 organisms into the conjunctival sac results in infection. Oral ingestion of organisms does not play a very big role in transmission.

Transmission of hardjo-bovis is very efficient: shedders are continually present in a herd and can transmit infection to other animals directly or indirectly through

contamination of the environment. Chronic urinary shedding reduces the importance of ponds or swampy pastures for survival of the organisms in the herd. The organism survives in carrier cows and can readily spread in dry climates from one herd to the other through the purchase of carrier animals.

Impact on Reproductive Performance

Strong evidence has accumulated linking *L. hardjo-bovis* infection with reproduction losses in beef and dairy herds. Experimental infection of pregnant cattle with hardjo-bovis has resulted in abortion,⁴⁴ stillbirths¹⁰ and weak calves.^{10,44} There have been numerous reports in the North American veterinary literature associating natural infection with hardjo-bovis in beef or dairy herds with repeat breeders,^{16,22} low pregnancy rates,¹⁷ abortions,^{22,36,37,40,42,44} stillbirths,²² or weak calves.^{17,22,44} Leptospirosis varied from the third to the sixth most commonly diagnosed cause of abortion in seven US veterinary diagnostic laboratory surveys reported over the past 40 years.³ Most recently, leptospirosis (all serovars combined) was the third most common diagnosis in abortions of beef or dairy cattle in California that were investigated from 1998 to 2003.³ Leptospirosis was diagnosed more frequently than bovine viral diarrhea virus, which came in fifth in that survey which established an etiologic diagnosis in 44% of 2,296 abortions.

In addition to abortions, stillbirths and weak calves, infection of cattle by *L. hardjo-bovis* has been shown to result in impaired conception or embryonic death. Reproductive performance measures were monitored for first-lactation cows on a California dairy that were either seropositive or seronegative to *L. hardjo-bovis* within 40 days after calving.¹⁶ Median time from calving to conception for seropositive cows (132.6 days) was significantly longer than for seronegative cows (95.4 days), and services per conception for seropositive cows (3.4) were significantly higher than that for seronegative cows (2.1).

Thus, the entire spectrum of losses due to reproductive tract pathogens results from hardjo-bovis infection: early embryonic deaths, abortions, stillbirths and weak calves. In addition, involvement of the mammary glands in hardjo-bovis infections can result in the "milk drop syndrome" of dairy cattle or "mastitis/flabby bag syndrome" of beef cattle.^{17,22,37,42} Mastitis of the dam has the potential to reduce weaning weights, or in severe cases, cause weak calves.

Beef herds generally harbor hardjo-bovis as an endemic infection, with minimal observable clinical signs such as sporadic abortions. Abortions are more common when a naive herd is initially infected with hardjo-bovis. Embryonic deaths, which are often not

recognized, may be the most damaging losses resulting from hardjo-bovis infection. Reproductive manifestations of hardjo-bovis infection will be greatest in replacement heifers because of their naiveté.¹⁷ This is true of all reproductive tract infectious disease. Another general principle of reproductive tract infections is that herd losses will usually be cyclic. There will be a year with substantial losses followed by several years of minimal losses. This happens because in the year of the heavy losses a very high percentage of the cattle become exposed and develop a protective immunity. For the next 2 to 3 years, the majority of females in the herd will be immune, therefore creating a "herd immunity" barrier against exposure of the non-immune, younger animals to the agent. Another cycle of disease can occur when the susceptible, unexposed females reach breeding age. Of course each cattle herd and its environment are different from other herds and even from itself from year to year, so many variations of disease patterns can occur.

Diagnosis

The definitive diagnosis of leptospiral infections has been culture and serologic identification of the infecting organism.³⁹ Culture of leptospira organisms is generally not used today because it is expensive and requires lengthy incubation. Serology, which is very useful for diagnosis of incidental leptospira infections, can give false negative or false positive results when used to detect hardjo-bovis infection. False negative serologic results can occur because cattle infected with hardjo-bovis mount a weak serologic response that peaks in 2 to 3 weeks, and falls below 1/100 or even negative by 4 or 5 weeks.⁷ Antibody titers in a dam that has aborted or has a weak calf must be > 1/800 to be diagnostic, and are commonly low enough at abortion to be indistinguishable from vaccination titers or even absent because of the one to three month interval that usually occurs between initial infection and abortion. False positive serologic results can occur because cattle naturally exposed to hardjo-bovis prior to vaccination with older leptospira 5-way vaccines can develop persistent microscopic agglutination test (MAT) titers to hardjo-bovis as high as 1/3,200 at six months after vaccination.⁴¹

Diagnosis in an individual cow can be made by combining serology with identification of leptospira in urine.^{4,28} Serum MAT titers to five leptospira serovars (pomona, hardjo, grippityphosa, icterohemorrhagiae and canicola) are measured, and urine is examined for spirochetes by fluorescent antibody (FA) or PCR.⁴ Clear urine is collected after administration of furosemide.³² Furosemide has been shown to facilitate enhanced recovery of leptospira. A cow is considered positive for hardjo-bovis when leptospira organisms are detected in

urine and serology is suggestive of hardjo-bovis infection (low titers to all serovars or a titer to hardjo-bovis higher than that expected from vaccination).

Diagnosis of herd infection can be made by the same combination of serology and detection of leptospire in urine in a subset of cows.^{4,28} It is recommended that 15 cows per herd be sampled in order to have a high probability (95% chance) of detecting one positive cow in a herd with a 20% individual animal infection rate. It is best to target cows for sampling that are found open at pregnancy examination or have a history of poor reproductive performance. A herd is considered infected with hardjo-bovis when one or more cows has leptospire in its urine accompanied by a serologic profile compatible with hardjo-bovis infection.

Diagnosis in an aborted fetus is usually made by demonstration of organisms in fetal tissues, placenta or urine by special stains, FA or PCR tests. Tissues that should be examined include kidney, liver and lung. Although fetal serology can sometimes help diagnose the cause of abortions, it seems to be of little to no value for diagnosing leptospiral abortions. Kirkbride observed no correlation between positive MAT titers in fetal serum and demonstration of organisms in fetal tissue.²⁴ Leptospira organisms were not identified by FA tests in tissues of 52 aborted fetuses that had positive MAT titers, while the MAT was negative in 15 aborted fetuses where leptospiral organisms were detected. In addition, causes of abortion other than leptospira were found in 18 of the 52 fetuses with positive MAT titers. When considering these discouraging comparisons, it must be remembered that leptospire are difficult to detect by FA in autolyzed tissues, and are easy to miss because of their irregular distribution in tissues. Causes of abortions in cattle are so difficult to diagnose that my standard recommendation for sampling strategy is to forward to the diagnostic laboratory, on ice, the entire fetus, pieces of placenta or a caruncle extracted from the uterus, and serum and urine from the dam.

Although the combination of identification of leptospira organisms in the urine by FA and identification of their serovar by evaluation of the serologic profile to five leptospiral organisms works well to diagnose hardjo-bovis infection, it has its pitfalls. The difficulty of interpretation of serum titers, especially in vaccinated animals, is an example. Recent research on diagnostics for leptospirosis has focused on development of enzyme-linked immunosorbent (ELISA) tests and PCR tests.^{34,39} A test that can identify serovar specific organisms in urine is needed. In the past, PCR tests on urine have been non-serovar specific. Some laboratories are now developing PCR tests to identify specific serovars of leptospira in urine.⁴⁶ The future gold standard test for leptospirosis is likely to be a urine serovar-specific PCR test with high sensitivity and specificity.

Control Programs

Decisions on implementation of a hardjo-bovis control program

The four key considerations on whether to implement a control program for a disease in a livestock operation are:

- Degree of disease threat.
- Amount of potential economic loss.
- Efficacy of control program.
- Cost of control program.

Unfortunately, veterinary practitioners often must give recommendations based on only partial information for some of these factors.

The chronic renal carrier animal that sheds organisms directly to other cattle and into the environment makes risk of introduction of hardjo-bovis into a beef herd higher with the following practices: purchase of replacements who are carriers, co-mingling with other herds of cattle for grazing, use of leased bulls, and access to a water course used upstream by other cattle.¹³ Herds that have a large number of cattle coming and going are at higher risk than closed herds. Threat of infection is high for ranches that have poorly maintained fences that allow neighbor cattle to mix with the herd.

Region of the United States definitely affects the degree of threat of leptospira infections of cattle. In a survey of 5,111 cows sampled at slaughter in 49 states, the percentage of cattle serologically positive for a leptospira serovar was much higher in southern tier states and west coast states than other areas of the US.³⁰ The percentage of cattle serologically positive for leptospirosis in the different regions of the US was Pacific coastal (65%), Rocky Mountain (44%), northern plains (37%), south central (60%), southeastern (60%), north central (34%), and northeastern (40%). Cattle were most commonly seropositive for hardjo-bovis. Authors of the study found that the geographic prevalence of leptospirosis was related more to annual temperature than to annual rainfall. Leptospira exposure was much more common in regions with a higher mean annual temperature. There are two genotypes of hardjo-bovis; hardjo-bovis A and hardjo-bovis B. Both types were isolated from the kidneys of cows in the southern states, while only hardjo-bovis A was isolated from cattle in the rest of the states.

We do not yet have an understanding of the amount of economic loss caused by hardjo-bovis in beef cattle herds. We do know that the type of reproductive impairment such as embryonic death and abortion it is associated with can severely lower the productivity and profitability of a beef herd. Its greatest threat to profits is hidden to most ranchers—early embryonic deaths.

Some clinical trials in other countries have stud-

ied the effectiveness of hardjo-bovis control programs in beef herds. The combination of antibiotic treatment and vaccination was successful in preventing new cases of hardjo-bovis in a large, closed beef herd in Scotland.²⁷ Abortion rates were reduced from 3.6% of non-vaccinated cows to 1.4% of vaccinated cows in a study conducted over a 6-year period in an Australian experiment station beef herd.¹⁸ There were no differences in rates of stillbirths, losses 48 hours after birth to weaning, or in weaning weights in progeny of vaccinates and non-vaccinates in that study.

The control program for hardjo-bovis discussed in this paper is based on sound evidence and should be successful. It is working excellent in a purebred beef herd that I oversee. This herd had abortions and stillbirths due to hardjo-bovis, as well as several other risk factors for abortion. At this time, there are no studies on the efficacy of hardjo-bovis control programs in the US similar to the two discussed above. Obviously, we need clinical trials in the US that utilize controls and vaccinates within the same herd to measure the efficacy of hardjo-bovis control programs.

The bottom line is that we have pretty accurate information about hardjo-bovis on the first and last considerations about whether to implement a control program (degree of disease threat and cost of control program). We are less sure of the two middle considerations (amount of potential economic loss and efficacy of control program). Considering all available evidence, the benefit:cost ratio of a hardjo-bovis control program is likely to be positive, over time, if a herd already has hardjo-bovis or is at high risk for infection. Thus, the most important factor bearing on a decision to implement a control program for hardjo-bovis is: how high is the degree of disease threat?

Enhancement of general herd resistance

Beef herd management programs designed to control infectious reproductive diseases are made up of management practices that foster a high level of general resistance against infectious agents combined with specific management practices aimed to prevent a particular disease. It's of critical importance for success that a hardjo-bovis control program is implemented in a herd that is already healthy with a high level of general resistance.

General resistance in a beef herd is promoted by a proper nutrition program, by minimizing stress to animals, by control of internal and external parasites, and by control of infectious agents that are immunosuppressive, such as bovine viral diarrhea and bovine leukosis viruses. The best vaccine in the world will fail to protect if management to enhance the general resistance of the herd is lax, such as a failure to provide a salt/trace mineral supplement designed to correct deficiencies

of minerals that are necessary for a strong immune response. There are many ways to minimize stress, such as avoiding overcrowding. Parasites deplete the body of protein and upset normal metabolism of protein which is needed for antibody production. Specific management practices to prevent hardjo-bovis infection include biosecurity, antibiotic treatment to remove the renal carrier state, and vaccination.

Management of purchased animals

Biosecurity - Biosecurity encompasses all the management practices used to prevent the introduction of a new disease into a herd. General principles of herd biosecurity are to insure that the ranch has fences adequate to keep potential carrier animals on the neighbor's ranch from mixing with the herd, only purchasing animals from well-managed herds with complete vaccination programs, only purchasing animals that are test-negative for the carrier state of unwanted diseases, and using a quarantine period to observe new purchases for signs of infectious disease that they may have been incubating.

Antibiotic treatment to eliminate the carrier state - During a quarantine period of 30 to 60 days, specific biosecurity practices can be taken to prevent introduction of hardjo-bovis into the herd. Presently, it's probably more practical to treat all quarantined animals with antibiotics to eliminate hardjo-bovis from the kidneys of potential carrier animals than to test for the carrier state and treat selectively. Testing is expensive, and there is considerable likelihood that some of the purchased animals will be carriers. In the past, dihydrostreptomycin has been used to eliminate chronic renal infection with leptospira. Recent studies have been conducted to find a more-suitable antibiotic for treatment of the renal carrier state of hardjo-bovis because dihydrostreptomycin is no longer available for use in food animals in the US, and it was not 100% effective anyway.^{1,38} Long-acting oxytetracycline^a given once at the label dose of 9 mg/lb (20 mg/kg) intramuscular (IM) stopped urinary shedding of hardjo-bovis in experimentally infected cattle.¹ No leptospire were detected in urine six weeks after treatment. This antibiotic was not as effective for eliminating the carrier state of hardjo-bovis when administered at a dosage of 5 mg/lb (11 mg/kg). In the same study, no leptospire were detected in urine at 4 to 6 weeks following treatment when ceftiofur^b was administered daily for five days at the label dose of 1 mg/lb (2.2 mg/kg) IM. Different treatment regimens with ceftiofur were not as effective. Clearance of any chronic infection by antibiotic treatment is a big order and not 100% effective. Thus, it's very important to adhere to the treatment regimens that were most effective in eliminating the renal carrier state.

Vaccination- Primer and booster vaccinations should also be given during the quarantine period. In 2003, a mono-valent vaccine of *L. hardjo-bovis* (Spirovac^{TMc}) became available in the US. Spirovac^{TMc} is now available as a multivalent lepto-5 vaccine (SpirovacTML5). Schering-Plough Animal Health has acquired the monovalent SpirovacTM and markets it as LeptoBovHB^{®d}. Following the introduction of SpirovacTM and LeptoBovHB[®], two other leptospiral vaccines, VL5 SQ^{®e} and Vira Shield[®] 6+L5^{HBf} have been introduced in the US with claims of protection against hardjo-bovis.

One of the greatest challenges that we grapple with daily as practicing veterinarians is to maintain the knowledge base needed to give our clients disease control advice that reflects the most recent scientific advances, evidence-based medicine. Even though I am constantly impressed with how diligent our profession is in continuing education activities, it's especially difficult for us to be confident and comfortable on our advice on use of vaccines. It's not possible for a veterinary clinician to keep abreast of all the numerous research pub-

lications on cattle vaccines that are published in multiple journals each year. A summary of the features of these new leptospiral vaccines would be useful to the busy practitioner. They have differences in composition and research data available to support claims of protection. Table 1 lists the vaccines and some of their features.

An immunologist's measure of the true efficacy of a vaccine is the amount of disease reduction in vaccinates, the preventable fraction (PF). The PF is the percentage of controls experiencing disease minus percent of vaccinates experiencing disease divided by percent of controls that experience disease.⁴⁵ Good, effective vaccines have a PF of at least 80%. Vaccinates must be challenged naturally or experimentally by a virulent pathogen and outcomes measured to obtain the PF. There is presently no data available on studies that compare reproductive performance in controls vs. vaccinates for any of the new vaccines that claim protection against disease losses caused by hardjo-bovis. There are, however, useful data on the abilities of some of these new

Table 1. Some important features of vaccines available in the United States with claims of protection against *Leptospira hardjo-bovis*.

	Vaccine			
	Spirovac ^{TMc}	LeptoBovHB ^{®d}	VL5 SQ ^{®e}	Vira Shield ^{®f} 6+L5 ^{HB}
Company	Pfizer Animal Health	Schering-Plough Animal Health	Intervet	Novartis Animal Health
Organism	<i>L. hardjo-bovis</i>	<i>L. hardjo-bovis</i>	<i>L. hardjo-prajitno</i>	<i>L. hardjo-bovis</i>
Origin of organism	Australia	Australia	Europe	United States
Available in multivalent lepto vaccine	Yes	No	Yes	Yes
Cell-mediated immunity	Strong, published in <i>Inf&Imm</i> ³¹ and <i>Vaccine</i> ¹²	Strong, published in <i>Inf&Imm</i> ³¹ and <i>Vaccine</i> ¹²	Studies in progress	No data
Protective against renal colonization	Yes, published in <i>Amer J Vet Res</i> ⁵	Yes, published in <i>Amer J Vet Res</i> ⁵	Yes, company data	No data
Protection against genital tract colonization	Yes, presented at <i>XXI World Buiatrics Congress</i> ⁶	Yes, presented at <i>XXI World Buiatrics Congress</i> ⁶	No data	No data
Protection of calves vaccinated at 4 weeks of age	Yes, published in <i>Aust Vet J</i> ³⁵	Yes, published in <i>Aust Vet J</i> ³⁵	No data	No data
Duration of immunity	12 months, data on file, APHIS, USDA	12 months, data on file, APHIS, USDA	No data	No data

vaccines to prevent establishment of infection. An effective hardjo-bovis vaccine must be capable of preventing the renal and reproductive tract carrier states. Vaccination with Spirovac™ prevented renal colonization and urinary shedding in heifers challenged with hardjo-bovis,^{5,6} and colonization of the uterus and oviducts.⁶

There has been much interest in the type of immune response elicited by Spirovac™, the first vaccine capable of protecting cattle against the renal carrier state caused by hardjo-bovis. Immunologists were startled by the results of their studies. Traditionally, humoral immunity has been considered the main defense against extracellular bacteria. That is so for most leptospiral infections, but pentavalent vaccines containing hardjo-prajitno and experimental hardjo-bovis vaccines failed to protect against renal colonization following challenge with hardjo-bovis, even though vaccines developed high concentrations of antibody.⁹ Recently, it has been shown that when initially confronted with an antigen, the immune system of mammals makes a decision to mount either a primarily cell-mediated response (type 1) or primarily humoral response (type 2).³¹ There is antibody production in both immune reactions, with emphasis on IgG2 production in a type 1 response and IgG1, IgA and IgE production in a type 2 response. Spirovac™ is unique because it induces a very strong cell-mediated immune response accompanied by IgG2 production, a type 1 immune response.^{12,31} It is speculated that hardjo-bovis may be more resilient to immune-mediated killing than other serovars of leptospira, and require a special immune response.¹² Antibody as well as cell-mediated immunity, however, must be important to resistance against hardjo-bovis infection because cessation of leptospiruria in 20 heifers experimentally infected with hardjo-bovis was invariably associated with a sharp increase in anti-leptospiral antibodies in the urine.²⁶

Management of the herd

Control of hardjo-bovis within the herd is accomplished by antibiotic treatment to eliminate the carrier state and vaccination. The program is designed to insure that carrier animals are not present in the herd, and that cattle in the herd have a protective degree of immunity against hardjo-bovis.

Successful control begins with young replacement heifer calves. Calves can be born infected because of *in utero* exposure to hardjo-bovis, and as calves born non-infected get older they are more likely to become infected due to post-natal exposure. Thus, as young as possible, calves should be treated with antibiotics to eliminate the possible carrier state and given their primer vaccination against hardjo-bovis. In beef herds, this could be done at first working and followed with a booster

vaccination booster 4 to 6 weeks later. A clinical trial utilizing a bivalent hardjo-bovis/pomona leptospiral vaccine with the same adjuvant and hardjo-bovis strain as Spirovac™ demonstrated that vaccination in the face of maternal antibody as early as four weeks of age with a booster in four weeks protected calves against experimental challenge with hardjo-bovis six months later.³⁵ If not possible to begin the hardjo-bovis control program at first working, it can be started at weaning, followed by booster vaccinations four weeks later. Either way, a second hardjo-bovis booster should be given to replacement heifers along with pre-breeding vaccines one month prior to breeding. Thereafter, they should receive an annual booster with their pre-breeding vaccinations. The importance of early embryonic deaths to losses caused by hardjo-bovis makes pre-breeding the most important time to vaccinate.

The first year of a hardjo-bovis herd control program, all yearlings and adults must be treated with antibiotics to eliminate the carrier state and given their primer vaccination for hardjo-bovis. Four to six weeks later, they should receive their booster vaccination. Thereafter, they should receive an annual booster with their pre-breeding vaccinations. An additional booster at pregnancy examination may be necessary, depending on vaccine used and degree of challenge within the herd.

Endnotes

- ^a Liqueamycin LA-200® - Pfizer Animal Health, New York, NY
- ^b Naxcel® - Pfizer Animal Health, New York, NY
- ^c Spirovac™, Pfizer Animal Health, New York, NY
- ^d LeptoBovHB®, Schering-Plough Animal Health, Princeton, NJ
- ^e VL5 SQ®, Intervet, Millsboro, DE
- ^f Vira Shield® 6+L5^{HB}, Novartis Animal Health, Larchwood, IA

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